MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XXVII SEPT.–OCT., 1935 No. 5

THE DEVELOPMENT OF CALVATIA CRANIIFORMIS

DELBERT SWARTZ [1]

(WITH 10 TEXT FIGURES)

It has been repeatedly pointed out by various workers (**1**, **2**, **3**, **4**, **8**, **13**, **14**), that the taxonomic arrangement of species of fungi should rest firmly upon facts gained from a study of the detailed development of as many species as possible. In previous papers the writer (**13**, **14**) has reviewed the literature dealing with studies of the development of species of Lycoperdaceae. It is hoped that the following report of studies of *Calvatia craniiformis* (Schw.) Fries may add something of value to our knowledge of relationships in the Lycoperdaceae.

MATERIAL AND METHODS

In October, 1933 the writer obtained a complete developmental series of fruit bodies of this species. The material was killed in Flemming's weaker solution and imbedded in paraffin in the usual way. For the younger stages entire fruit bodies were used; in the larger fruit bodies sections of various parts were imbedded. Sections were cut 7 μ thick, and stained in Heidenhain's iron-alum haematoxylin.

[1] Research paper, Journal Series, University of Arkansas, *No. 363.*

[MYCOLOGIA for July–August (**27**: 333–438) was issued August 1, 1935]

OBSERVATIONS

Description of fruit body: Fruit body very large, obovoid or turbinate; base stalk-like, thick, stout with a cord-like root; peridium thin, papery, smooth, pallid or grayish, breaking up into uneven areas at maturity and falling away. Subgleba honeycombed, spongy, concave at top, and long time persistent. Gleba at first white, becoming greenish yellow to ochraceous at maturity. Capillitial threads slightly branched, sparsely septate, about as thick as the spores. Spores spherical, smooth with a short pedicel 2.8–4 μ in diameter.

Rhizomorph: The rhizomorphs of *Calvatia craniiformis* are well developed, and for the most part are similar in structure to those of other species of Lycoperdaceae (**13**). In longitudinal section there are three layers: (1) Outer cortex layer, (2) Subcortical layer, (3) Central core. The outer cortex layer is composed of relatively thick, blunt, interwoven hyphae which stain more deeply than do the hyphae of the other layers. They arise from the more compact subcortical layer, but in contrast to them they have no visible internal differentiation. This seems peculiar because the hyphae of the outer layer are connected to living hyphae of the layer just beneath. At the tip of the rhizomorph this cortex makes up the greater part of the total diameter.

The subcortical layer is very thin and is composed of hyphae which for the most part are parallel to the long axis. There is comparatively little or no interweaving. These hyphae have thin walls and do not stain deeply. On the inside they are continuous with the larger hyphae of the central core.

The component hyphae of the central core are usually several times as thick as those of the subcortex. Septa occur frequently. There are not as many large deeply staining crystals as have been seen in other species (**13**, **14**). Hyphae of the subcortical layer may enter into this central core, continue in the larger size and shape, and then enter once more into the subcortex where they assume the size characteristic of the hyphae of that region. The most conspicuous feature of the rhizomorph is the extremely large size of the hyphae of the central core; *Lycoperdon pedicellatum* Peck (**14**) is the only investigated species in which the size is comparable.

When a rhizomorph of *Calvatia craniiformis* branches, the resulting branch arises chiefly from the hyphae of the central core and not from the hyphae of the subcortex (FIG. 1).

Formation of the fruit body: The fruit body arises from the tip of a rhizomorph or from a lateral branch of it. This species is similar in this respect to other investigated Lycoperdaceae. Young fruit bodies are surrounded by hyphae resembling those of the cortex of the rhizomorph, but the homogeneous interior is made up of hyphal branches originating in the central core.

Peridium: The peridium in this species is similar to the same structure in *Calvatia saccata* (**13**). There is no division into the exoperidium and the endoperidium; instead this outer layer becomes pseudoparenchymatous at an early age. The hyphae do not become arranged radially parallel as in other species. This arrangement is brought about by the interweaving of the tangential hyphae, and is somewhat similar to the layer of pseudoparenchyma in *Bovista plumbea* Pers. (**13**). The development of this layer is more complete in *Calvatia craniiformis* than in any other species (FIG. 2, 3, 4). On the periphery there are a few loosely scattered tangential hyphae; they are present in greater numbers at first, but seem to wear away gradually. In the younger fruit bodies these hyphae are simply extensions of the cortical layer of the rhizomorph, while in the more mature fruit bodies they are the outermost extensions of those of the pseudoparenchyma. This is further reminiscent of *Bovista plumbea* but as will be shown the two layers are really quite different. Between the pseudoparenchyma (FIG. 3) and the gleba is a region of somewhat elongated flattened cells (FIG. 4, 5). These cells are not differentiated into any structure which can be compared to the endoperidia of other species; instead it simply marks the extension of the hyphae of the gleba into the pseudoparenchyma. There is no separate layer formed; the hyphae are connective. The flattening is due to pressure on the inside due to the rapidly expanding gleba.

Gleba: The gleba is formed in the same general way in *Calvatia craniiformis* as in other investigated species (**13**). In very young fruit bodies it is composed of a homogeneous mass of hyphae which are continuous with and very similar to the hyphae of the central core of the rhizomorph. As growth continues these hy-

phae become more closely compressed because of the extremely active branching of the original threads. Although the fruit bodies are ordinarily quite large when mature, glebal differentiation begins at a very early age. Cavities are formed and become lined with their characteristic even hymenium when the fruit bodies are quite small. A few scattered spores have been seen soon after the first hymenial layers have been laid down. This has been reported in other species (**12, 13**). The presence of spores at such an early age is significant; it simply indicates that the stage of glebal development can not be correlated with the size of the specimen in question. These results differ from some reports of other investigators (**9**). In addition to the hymenial layer, there is a tramal layer and a subhymenial layer found in developing fruit bodies (FIG. 6). The trama is composed of large, septate, multinucleate hyphae which are rather closely compressed and form a very pronounced strand which winds in and out between the rapidly forming cavities (FIG. 6). The tramal hyphae branch freely on either side to form subhymenial layers. These layers of subhymenia are rather poorly developed in this species, and when present appear rather late. When they are absent the basidia arise directly from the trama (FIG. 7).

Additional cavities are formed rapidly in young fruit bodies by a mechanical separation of the hyphae of the trama. They enlarge in two ways:—(1) by the insertion of new basidia and (2) by the coalescing of neighboring cavities (FIG. 6, 7). Frequently large, conspicuous hyphae arise in the trama, pass through the hymenial layer, through the cavity, and are lost in the hymenial layer on the opposite side of the cavity. Such threads are evidently tramal hyphae which have not been pushed apart during the formation of the cavity, and are often seen in various stages of disintegration in fruit bodies of more mature age. The gleba is always more completely formed in the apical portion, and matures there first. The spores are attached to the basidia by sterigmata and are first visible as terminal swellings on them.

At the base the gleba is joined to the sterile base (FIG. 9). Ordinarily the hyphae at the region of connection become rearranged forming a horizontal layer. This rearrangement permits the complete wearing away of the spores and capillitium without dis-

turbing the sterile base. It is suggestive of the columella described by some workers (**12**) in other species.

The details of nuclear behavior were not studied.

Capillitium: The capillitium in *Calvatia craniiformis* is formed in the same way as described for related species (**12, 13, 14**). In this species the first formed capillitium is visible near the connection of the peridium and the gleba, but later it is of general occurrence throughout the trama.

Sterile base: The very well developed sterile base appears some time after the differentiation of the gleba has begun. In my specimens the hyphae of the sterile base are not so well organized as in certain other species (**13**). They are at first loosely interwoven and gradually assume the arrangement reported in similar structures in related species (**13**). As was pointed out above the sterile base is separated from the gleba by a fairly well organized layer of hyphae (FIG. 9). When the fungus has matured and begins to dry out this layer evidently becomes completely formed; it is not unusual to see sterile bases still attached to the ground long after the glebal mass has completely disappeared.

Dehiscence: The peridium gradually dries and falls away in flakes, thereby exposing the mature spore mass. Sometimes the entire peridium is broken up before any of it falls away. Just why the peridium should break up as it does is not clear; there are no indications of the formation of weakened hyphal zones.

DISCUSSION

The results of this study of *Calvatia craniiformis* show that the developmental processes proceed along the same general lines previously reported for other investigated Lycoperdaceae. The additional findings indicate once more that related species have their own characteristic mode of development. These developmental differences, as might be expected, indicate hitherto unsuspected relationships.

The peridium which has been ordinarily considered two-layered in the Lycoperdaceae, here, as in the case of *Calvatia saccata* (Fries) Lloyd, is shown to be composed of only one well-defined layer. A thin layer of adhering cortex hyphae and the ends of

some of the peridial hyphae make up the periphery of the peridium (FIG. 10). There is no complete, permanent extension of this layer around the upper globose part of the mature fruit body; hence it cannot be considered as a separately differentiated peridial layer. The differences that appear in the hyphae of the peridium proper are due to external changes resulting from internal pressure caused by the expansion of the gleba. Naturally this layer, subjected to pressure from the inside only, shows the results of this by a somewhat looser arrangement at the periphery (FIG. 5). This outer layer is connected directly to the region just beneath where the cells have been subjected to additional pressure from the inside and have become definitely pseudoparenchymatous; they have been limited on the outside by the cells already there. Further this pseudoparenchyma is connected to hyphae which arise in the gleba (FIG. 4); they are not tangential in direction, not compactly compressed, nor do they form a definite layer which in any way compares to the endoperidium of the lycoperdons. This outer covering, the peridium (FIG. 10), is interpreted as a one-layered structure in which the cells at various distances from the periphery have become somewhat modified because of various growth tensions. In certain descriptions of the calvatias (**5, 10**) mention has been made of two layers; it seems interesting to note that the two investigated calvatias, *Calvatia saccata* and *Calvatia craniiformis,* show the same general make-up of the peridium. In the light of this evidence it seems safe to consider a one-layered peridium characteristic of the genus. It will be interesting to see just how general this condition is among the remaining uninvestigated species.

The peridium of *Calvatia craniiformis* shows still another somewhat unsuspected relationship. The outermost fringe of hyphae in *Calvatia craniiformis,* as well as the layer of pseudoparenchyma, is very similar in appearance to the two outermost layers found in *Bovista plumbea.* Sculpturing is not found on the exterior in either species. However, the development of this outermost layer of tangential hyphae in *Bovista* indicates a relationship of this species to *Geaster* (**13**). In *Calvatia saccata* the outermost region of the peridium is composed of adhering meal-like granules, and

these were considered the remains of any exoperidium it may have had (**13**). In *Calvatia craniiformis* the loosely arranged external layer may be interpreted in the same manner. The spines characteristic of the outer peridial layers in *Lycoperdon pulcherrimum* Berk. and Curt., *Lycoperdon Wrightii* Berk. and *Lycoperdon pyriforme* (Schaeff.) Pers. have been considered homologous with the outer tangential region in the very well-developed peridium of *Bovista plumbea*; there seems to be nothing in either of our calvatias which can be considered homologous to these structures. This opinion is held in spite of the loosely adhering granules in the one and the tangential hyphae in the other (FIG. 10). It is evident that these are simply related to a small part of the exoperidium of the others.

The gleba in this species, during its differentiation and subsequent development is essentially the same as in the other investigated species of Lycoperdaceae. Here, as elsewhere, the hymenium arises from the trama (FIG. 8). The trama of this species as in *Calvatia saccata* is well developed (FIG. 6). The subhymenial layer, however, is poorly developed; this is similar to the condition found in *Lycoperdon Wrightii* and *Lycoperdon pulcherrimum*. However, in *Calvatia saccata* the subhymenium is well developed. In some specimens of *Calvatia craniiformis* the hymenium branches directly from the trama; in others a definite subhymenium is formed. This unexplained variability can not be relied upon to furnish conclusive evidences of relationship or lack of it.

Cavity formation, as well as spore formation, is similar to the other investigated Lycoperdaceae. Capillitium is formed in this species from living hyphae and seems to function in the disposal of waste material. The only difference is in the appearance of the first threads in the region of the connection of the gleba and peridium; this has not been observed by the writer in other species. It has, however, been reported by Cunningham (**6**) and by Rehsteiner (**12**). This condition is only temporary because the capillitium threads appear in greater numbers in the region of the trama as the fruit body undergoes the later stages in its development. Aside from these facts the structure of the gleba is similar to other species.

The rearrangement of the hyphae at the region of the union of the gleba and the sterile base is somewhat similar to that observed by the writer (**14**) in *Lycoperdon pedicellatum* Peck and by Miss Rabinowitsch (**11**) in *Lycoperdon depressum* Bon. However, it scarcely seems plausible to consider this line of separation as a sufficiently differentiated structure to be designated a columella. On the other hand, if we are to consider such structures columellae regardless of how well or how poorly they are developed, then certainly *Calvatia craniiformis* has a columella. Such an interpretation seems undesirable; it seems that the sense of 'grenz-linie' as used by Miss Rabinowitsch is more satisfactory.

The sterile base of *Calvatia craniiformis* comprises about half of the entire fruit body at maturity. The well developed sterile base is suggestive of a relationship to the majority of the lycoperdons. However, this characteristic seems to vary within the genus *Calvatia*. *Calvatia gigantea* Batsch. has comparatively little sterile tissue in proportion to its size. In *Calvatia saccata, Calvatia cyathiforme* Bosc. as well as in *Calvatia caelata* Bull. the basal portions are very well developed. This character can hardly be considered of importance in establishing relationships unless it can be shown that *Calvatia gigantea* does have a sterile base sometime during its development.

The method of spore dispersal in our species is typical of the known calvatias. It differs somewhat from the condition described for *Calvatia saccata* which at times has an apical opening similar to the lycoperdons. This condition has not been observed in *Calvatia craniiformis*. Although the peridial hyphae become rearranged at the time of pore formation in the lycoperdons and the geasters, no such changes have been seen to account for the uneven breaking up of the peridium of the calvatias.

The large rhizomorph of this species is similar to the other investigated species and differs only in the poor development of the subcortical layer and the extremely large hyphae of the central core. These characters do not seem significant.

The above observations indicate that additional studies of the development of other species of *Calvatia* will prove of value and

will aid in the ultimate understanding of relationships in the Lycoperdaceae.

The writer wishes to express his thanks to Dr. D. M. Moore for the collection of this material.

UNIVERSITY OF ARKANSAS,
FAYETTEVILLE, ARKANSAS

LITERATURE CITED

1. **Atkinson, G. F.** The development of *Agaricus arvensis* and *Agaricus comtulus*. Am. Jour. Bot. **1**: 3–22. 1914.
2. ——. The development of *Lepiota clypeolaria*. Ann. Myc. **12**: 346–356. 1914.
3. ——. The development of *Amanitopsis vaginata*. Ann. Myc. **12**: 369–392. 1914.
4. ——. The origin and development of the lamellae in *Coprinus*. Bot. Gaz. **61**: 89–130. 1916.
5. **Coker, W. C. & J. N. Couch.** The Gasteromycetes of the eastern United States and Canada. 201 p. Chapel Hill, North Carolina. 1928.
6. **Cunningham, G. H.** The development of *Lycoperdon depressum*. New Zealand Jour. Sci. and Tech. **8**: 228–232. 1926.
7. ——. The development of *Geaster velutinus*. Trans. Brit. Myc. Soc. **12**: 12–20. 1927.
8. **Kauffman, C. H.** The genus *Clitocybe* in the United States, with a critical study of all the North American species. Papers Mich. Acad. **8**: 153–214. 1927.
9. **Lander, Caroline A.** The morphology of the developing fruiting body of *Lycoperdon gemmatum*. Am. Jour. Bot. 20: 204–215. 1933.
10. **Morgan, A. P.** North American Fungi. The Gasteromycetes. Jour. Cin. Soc. Nat. Hist. **14**: 170–171. 1892.
11. **Rabinowitsch, Lydia.** Beitrage zur Entwickelungsgeschichte der Früchtkörper einiger Gastromyceten. Flora **79**: 385–418. 1894.
12. **Rehsteiner, H.** Beitrage zur Entwickelungsgeschichte einiger Gastromyceten. Bot. Zeit. **50**: 761–878. 1892.
13. **Swartz, Delbert.** Some developmental characters of species of Lycoperdaceae. Am. Jour. Bot. **20**: 440–465. 1933.
14. ——. The development of *Bovistella pedicellata* (Peck) Lloyd. Am. Jour. Bot. in press.

DESCRIPTION OF FIGURES

Fig. 1, a branching rhizomorph (Note the large hyphae of the central core at base; also the three characteristic layers of the rhizomorph); 2, the

outermost tangential hyphae of the periphery of the fruit body; 3, extremely well developed pseudoparenchyma of the central part of the peridium; 4, the somewhat elongated, flattened hyphae connecting the gleba and the peridium; 5, the looser pseudoparenchyma of the peridium; 6, the well developed tramal layer between the cavities; 7, showing basidia arising directly from the trama; the coalescing of neighboring cavities; also a few sterigmata on the basidia; 8, the details of the hymenium and its relationship to the branches of the trama; 9, the region of union of the fertile gleba and sterile base; also the rearrangement of hyphae at the same point; 10, photograph of the entire peridium and the adjoining gleba; note the pseudoparenchymatous condition of the peridium.

SOME MISCELLANEOUS FUNGI OF THE PACIFIC NORTHWEST [1]

S. M. Zeller

(with 3 text figures)

This paper reports one genus and four species as new, two new combinations, four species new to America, and thirty-six new to Oregon. The types of the new species are deposited in the Herbarium of the Department of Botany, Oregon State College. Unless otherwise stated the localities referred to are in the state of Oregon. Ridgway's standard was used for describing colors.

PHYCOMYCETES

1. Dicranophora fulva Schr.

Parasitizing *Boletus,* Benton, Linn, and Jackson Counties. Common in October. Perhaps this is the first report of this fungus from western United States. Thaxter, however, found it parasitic on *Boletus* in Maine.

ASCOMYCETES

2. Valsa coronata Fries.

On twigs of *Castanopsis chrysophylla,* near Eugene, Lane County. December.

3. Poronia Oedipus Mont.

On horse dung, Corvallis. April.

4. **Leptosphaerulina Sidalceae** sp. nov.

Peritheciis nigris, oblato-sphaeroideis, subepidermicis erumpentibus, 300–400 μ crasso, 200–250 μ alto, ostiolo papillato; ascis octosporis cylindraceis, in pedicellum brevem attenuatis, 140–180 × 30–37 μ, aparaphysatis; sporidiis

[1] Published as Technical Paper No. 213 with the approval of the Director of the Oregon State Agricultural Experiment Station, Corvallis. Contribution from the Department of Botany.

distichis, ellipsoideis utrinque obtusulis, muriformiis 6- vel 7-transeptatis, fulgineis, 37–50 × 14–23 μ.

In caulibus emortuis *Sidalceae campestris,* Benton county, Oregon, Amer. bor. Aprilis.

FIG. 1. Canker of *Alnus rubra* caused by *Nectria ditissima* var. *major* Wr.

Perithecia oblate-sphaeroid, black, supepidermal, erumpent, 300–400 μ broad, 200–250 μ deep; *ostiole* papillate; *asci* 8-spored cylindrical, short-stipitate, 140–180 × 30–37 μ, aparaphysate; *spores* muriform with 6 to 7 transverse septa, fuliginous, 37–50 × 14–23 μ, broadly ellipsoid with blunt ends, usually 2-seriate.

On dead flowering stems of *Sidalcea campestris* Greene, North Benton County. April.

5. NECTRIA DITISSIMA Tul. VAR. MAJOR Wollenweber.

On *Alnus oregona* near the summit of the coast range on the McMinnville-Tillamook highway. Tillamook County. March. Collected by *L. N. Goodding.*

As the illustration (FIG. 1) shows, this fungus causes a canker of alder quite typical of the *Nectria* cankers of other hosts. So far as the writer knows, this is the first report of this form in North America, it having previously been found in Norway only. Ours is typical with ascospores 17.7–22 × 7–9.2 μ and the characteristic asci which have about 4 or 5 spores crowded near the upper end and 3 or 4 in a row below, as illustrated by Richter [2] and Wollenweber.[3]

6. HYSTEROGRAPHIUM FRAXINI (Pers.) De Not.

On *Fraxinus oregona,* Eugene-Lorraine Road. Lane County. March.

7. COCCOMYCES DENTATUS (Kunze & Schm.) Sacc. VAR. HEXAGONUS Penx. & Sacc.

On leaves of *Mahonia nervosa,* Alsea Mt., Benton County. March.

This collection is upon a different host than before reported but it answers the description for the above species and variety. The perithecia are nearly all hexagonal, epiphyllous and hypophyllous, shiny black, 1 mm. in diameter on nearly white spots. The asci are 90–100 × 7–9 μ, and the spores are linear to acicular, continuous, 27–36 × 1–2 μ.

8. ATROPELLIS PINICOLA Zeller & Goodding.

On living branches of *Pinus lambertiana,* Dorrington, Calaveras County, California, Aug. 16, 1930 (*Frank A. Patty*) and on *P. Strobus* in a planting, Mt. Hebo, Tillamook County, Oregon, Aug. 19, 1931 (*L. N. Goodding*) and July 23, 1932 (*L. N. Goodding* and *Geo. Root*).

[2] Richter, H. Die wichtigsten holzbewohnenden Nectrien aus der Gruppe der Krebserreger. Zeitsch. Parasitenk. 1: 24–75. *illus.* 1928. (See p. 48.)

[3] Wollenweber, H. W. Pyrenomyceten. Studien II. Angew. Bot. **8**: 168–212. *illus.* 1926. (See Taf. IV, 32.)

Pinus Strobus is a new host for this parasite and the collection on *P. lambertiana* extends the southern known range of the disease.

9. CENANGIUM PINIPHILUM Weir.

Parasitic on *Pinus albicaulis*, Taylor Burn, Lane County. Sept. 3, 1931 (*Conrad Wessela* and *L. N. Goodding*). This is a new host for this fungus.

10. CIBORIA AMENTACEA (Balb.) Fuckel.

On fallen male catkins of *Alnus rubra* Waldport. March.

11. BELONIOSCYPHA CILIATOSPORA (Fuckel) Rehm.

On stems of *Aster* (*A. novi-belgii* x *A. Douglasii*) which have been dead about a year. December.

12. PERROTIA FLAMMEA (Alb. & Schw.) Boud.

On acorns and cups of *Quercus garyana*, near Corvallis. May. This species has previously been reported west to Montana and Colorado.

13. RHIZINA INFLATA (Schaff.) Karsten.

Associated with and parasitic on roots of *Pinus contorta* in sandy hummocks near the ocean beach at Big Creek, Lincoln County. There were great quantities of the fruiting bodies on the sand covering an area about 30 feet in diameter. If this fungus is not parasitic it at least smothers seedlings 2 to 3 years old. This is the first report of this species west of Idaho.

14. LEOTIA LUBRICA Fries.

In heavy moss under conifers, Coos County. November.

15. MORCHELLA BISPORA Sorokin.

On the ground in mixed woods, near Peoria, Linn County, and Jefferson, Marion County. April.

These are trim, erect plants with nearly totally free caps. The asci are 2-spored, the spores measuring $74 \times 18.5\ \mu$. This species has previously been reported as far west as Minnesota.

16. DURANDIOMYCES PHILLIPSII (Mass.) Seaver.

On old burned straw stack, Tygh Ridge, 15 miles south of Dufur, Wasco County. November. Collected by *Dr. Roderick Sprague*.

17. ASCOPHANUS TESTACEUS Phillips.

Growing in a damp chamber on paper and sclerotia from lettuce which had been collected in Benton County. May.

BASIDIOMYCETES

18. GYMNOSPORANGIUM NELSONI Arthur.

On *Juniperus occidentalis* Nutt., Cove. Union County. April. Small galls on the branches are associated with the telia of this rust.

19. TRIPHRAGMIUM CLAVELLOSUM Berk.

On leaves of *Aralia racemosa* L. (American spikenard or Indian-root), Yoncella, Douglas County. July.

This is the first report of this rust from the Pacific Northwest.

20. **Clavaria brunnea** sp. nov.

Solitaria, 11–12 cm. alta, 7–8 cm. crassa; stipite distincto, profundo radicans, 1–2.5 cm. crasso, pallide brunneolo, basi pallide cremeo; rami plures, erecti, plerumque recti, cylindrici, interdum furcati, apicibus briviter 2–3-fidis, angulis acutis, superficie pulverlentis, "pecan brown" vel "fawn color," "army brown" vel "natal brown"; carne cremea vel flavescente, amarescente; sporis pallide brunneis, oblongis vel ellipsoideis, subverrucosis, 8–10 × 4–5 μ.

Ad terram inter folia dejecta praecipue Coniferarum, prope Seal Rocks, Lincoln County, Oregon, Amer. bor.

Among fallen leaves, especially of conifers, near Seal Rocks, Lincoln County. October 7, 1931. Collected by Mrs. Frank York.

Plants solitary, 11–12 cm. tall, 7–8 cm. broad, with a distinct, rather deeply rooted stem, 1–2.5 cm. thick; *branches* numerous erect, mostly straight, pencil-like, sometimes branched, tips usually short-forked, angles sharply acute; surface pulverulent; color of branches "pecan brown" to "fawn color" in lighter shades but "army brown" to "natal brown" where darker, drying "Buck-

thorn brown " (" mummy brown " where bruised), tips concolorous and stem lighter brown to almost creamy white in parts; *flesh* creamy to flavescent, slightly bitterish; *spores* light brown, oblong to ellipsoid, slightly roughened, 8–10 × 4–5 μ.

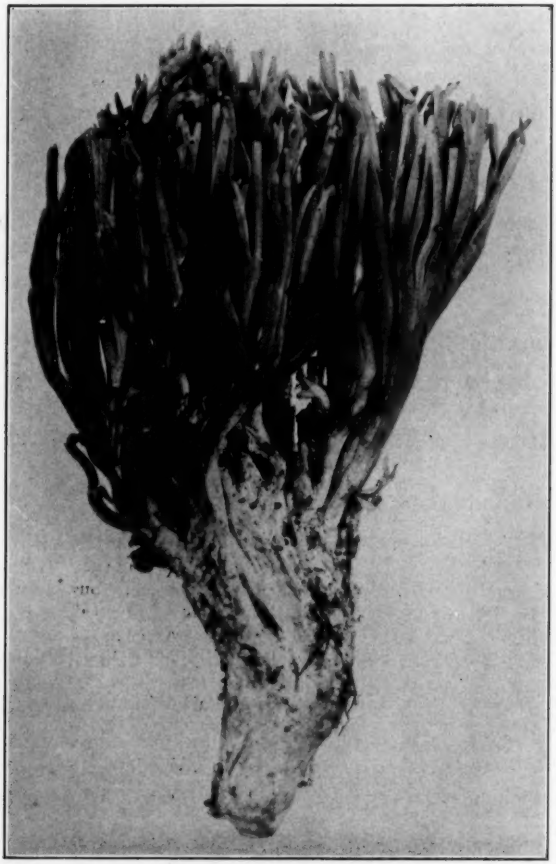


FIG. 2. *Clavaria brunnea.*

This species reminds one of *C. grandis* in color and size (FIG. 2) but it differs in size and shape of spores, the acute angles of the branches and it occurs in coniferous woods. The spores are merely

roughened while in *C. grandis* they are distinctly echinulate or verrucose.

21. CLAVARIA SANGUINEA Pers.

In coniferous woods, Lincoln County. November. Frequent.

This beautiful red *Clavaria* was previously reported by Coker from Massachusetts and North Carolina. The Oregon plants when fresh are pink to red throughout except the whitish base. These colors are mostly spinel pink to spinel red or alizarine pink on shaded sides.

22. CLAVARIA STRICTA Pers.

On alder wood from white rhizomorphs, Alsea Mt., Benton County. November. Frequent.

C. stricta has been found and recognized several times in recent years. This is the first report of the species from the Pacific slope. Coker, however, reported it from Idaho.

23. BOLETUS EASTWOODIAE (Murrill) Sacc & Trott.

Solitary, in clay soil under *Quercus garryana*, in the Starr Creek district, Benton County. October. (FIG. 3.)

Two specimens were found, one in fine condition but the other was badly decayed. Measurements of the older specimen were taken. I have referred the collection to *Boletus Eastwoodiae* because it agrees in the outstanding characters but differs in the shades of color. This may be due to the fact that the original description was prepared from dried specimens. I have not seen the type. The following descriptive notes are presented to augment the original description.

Plants solitary; *pileus* up to 18 cm. broad, thick, solid, surface soft glabrous, shining to innately-reticulate-areolate, pinkish buff to light olivaceous shades in the areolae, with pink shades as on the stipe; *context* white to yellowish, changing to blue when wounded or cut, *but soon* returning to the original color; *tubes* nearly free, (surely not "adnate"), 1–1.7 cm. long, scissile, colonial buff to olive-ocher becoming citron green to lime green when exposed, with bluish tinges when bruised, drying dark olive buff to buffy brown; *mouths* circular (not "angular") when fresh, brick red

when looking into tubes, but morocco red when looking across them. The tramal tissues are of hyaline, septate hyphae which are 5–6 μ in diam., with occasional filaments which appear like lactiferous ducts. The tubes easily split from the pileus context. *Basidia* hyaline, 4-spored, broadly clavate to pyriform, 24–28 $\times$ 10–

FIG. 3. *Boletus Eastwoodiae.*

12.4 μ; *sterigmata* hyaline, short, 3–4 μ long, slender; *spores* 10–14 $\times$ 4–5.5 μ, fusiform to narrowly ellipsoid, golden-yellow; *Cystidia* awl-shaped, rather sharp, granular, hyaline, 17–22 μ long; *stipe* 9–10 cm. long, 7–9 cm. broad in the middle, bulbose, solid, surface having yellowish under cast but mostly with pink tints (from orange-pink to light Corinthian red), flesh color with reticulations above.

The colored illustration of *Boletus purpureus* Fries (Atlas Pl. XII, Suppl. to Vol. 42, Bull. Soc. Myc. Fr. 1926) is very good for this collection except in the color of the pileus and flesh and reticulations on the stipe. It seems to have its closest relationships with *B. purpureus* Fries and *B. Queletii* Schulzer var. *rubicundus* R. Maire.

24. **Boletus olivaceobrunneus** Zeller & Bailey sp. nov.

Pileo 9–14 cm. lato, hemisphaeroideo vel convexo-expanso "olive-brown," centro leviter obscurioribus, vulnerata ad "fuscous-black" tarde mutante, aequo, sicco, breviter tomentuloso velutino spectans, leviter fissurato siccans; margine integro; carne usque 2.5 cm. crassa, firma vel spongiosa sordido-alba, cinerescens, sapore et odore miti foetide siccans; tubulis adnatis sinuatis interdum liberis fractans, primis subplanis avellaneis, demum depressis "snuff-brown" vel "bister," ore minute, 12 pro cm. circa, subrotundo, primo farcto, "buffy brown" vel "olive-brown"; stipite subcyclindrico deorsum incrassato, 12–15 cm. longo, superne 2–3 cm. crasso, inferne 3–4 cm. crasso, superficie pileo pallidioribus longitrorsum costatuloso, superne reticulato, costae pileo concolore, solido, intus fibrilloso-carneosos sordido-albido, vulnerato ad brunneo-nigricans; sporis fusiformibus lexibus, brunneo-ferrugineis, 14–15 × 5–6 μ, guttulatis.

Ad terram in silvis Coniferarum prope Siletz (*F. D. Bailey*) et Newport, Lincoln County, Oregon, Amer. bor. October et November.

Pileus 9–14 cm. broad, hemisphaeroid to convex expanded, olive-brown (R) somewhat darker at the disk, fuscous-black where bruised, even, dry, minutely tomentulose giving a plushy appearance, cracking slightly on drying; *margin* entire; *flesh* up to 2.5 cm. thick, firm to spongy, sordid white becoming drab on exposure; *taste* and *odor* mild, drying fetid; *tubes* adnate, sinuate, sometimes breaking free, at length depressed, nearly plane, avellaneous when fresh, changing snuff-brown to bister, *mouths* small, averaging 12 per cm., subcircular, stuffed when young, buffy brown to olive-brown; *stem* subcylindrical, tapering upward, 12–15 cm. long, 2–3 cm. diam. above to 3–4 cm. below, solid, fibrillose-fleshy, sordid whitish changing brownish black within, surface slightly lighter than pileus, longitudinally ribbed, ribs concolorous with pileus, anastomosing, reticulate above; *spores* fusiform, smooth, brownish-ferruginous, 14–15 × 5–6 μ, large guttulae.

Under *Picea sitchensis*, Siletz, October, *F. D. Bailey* (type); Newport, November, *Professor* and *Mrs. H. P. Barss.*

25. BOLETUS PINICOLA (Vitt.) Rea.

Under conifers, Parmelia Creek, Santiam National Forest, Linn County, May.

This is the first report of the species from Oregon, if not from North America. It answers the description perfectly, with the possible exception of the marginal white line which in ours shows merely as a semblance of a line in two or three short arcs.

26. BOLETUS RETIPES Berk. & Curt.

Under conifers near the Three Sisters in the Cascade Range, August. Collected by *W. E. Lawrence.*

The Oregon material answers the description in every way, except that the surface is cracked into truncated polygonal areolae as described for *B. frustulosus* Peck. This is not the first time this character has been described for *B. retipes,* however. This is perhaps the first time this plant has been reported west of Wisconsin.

27. FOMES ANNOSUS (Fries) Cooke.

On down logs of *Acer macrophyllum,* Alsea Mountain, Benton County. Infrequent but this record of the species on a hard wood is of interest. December.

28. POLYPORUS LAPPONICUS Romell.

On dead wood of *Pinus contorta,* on the slopes of Mt. Hood in Hood River County. August.

This plant has pronounced rosy tones so that one may at first feel that it can be referred to *P. fragilis* but it has much larger spores and differs in texture. I am taking the liberty to report this fungus under the name *P. lapponicus* Romell because of the following note from Dr. L. O. Overholts:

"Your No. 7546 would seem to be too near *P. ursinus* Lloyd (Synopsis Apus Polyporus, p. 319) to allow of separation. The species is common enough in the Rockies from Idaho to Colorado, but this is the first collection from west of the mountains. It also occurs in New England, New York, and Michigan, but apparently is a boreal species, going up to timber line in the Rockies. Some other name than *P. ursinus* will have to be applied to it, as it is not *P. ursinus* Link, from Brazil. *P. lapponicus* Romell is a synonym and probably the proper name for it."

29. POLYPORUS OVINUS (Schaeff.) Fries.

The following description is from fresh specimens:

Pileus circular to somewhat irregular, convex to plane or somewhat depressed at center, 5–9 cm. broad, 1–1.5 cm. thick at the

center, very thin at margins; *surface* even, tomentose at the center to squamulose appressed scaly, cream-buff to ochraceous-buff with dark reddish brown scales; *margin* very thin, even, somewhat undulating; *flesh* spongy, creamy white; *tubes* decurrent, 2–4 to the mm., angular, 1–2 mm. long, edges thin, fimbriate, straw yellow to empire yellow when fresh, cream color to wax yellow when drying, becoming deep olive buff to olive lake and deep Colonial buff when dry; *stipe* central sometimes excentric, equal, 3–8 cm. long, 0.7–2.0 cm. thick, usually a little lighter than the pores, sometimes with pinkish tints, staining brownish where bruised, solid with white flesh, smooth; *spores* smooth, hyaline, one large vacuole, broadly ellipsoid to subglobose, 4–4.5 × 3.5–4 μ; *odor* pleasant; *taste* mild.

In coniferous woods. Newport, October 18, 1931, *H. P. Barss.* Gregarious but rare.

This collection was identified by Dr. L. O. Overholts and consisted of 9 specimens. It is evidently a rare species, but widely distributed having previously been reported from Alabama, Colorado, and New York; and now from just above sea level in Oregon.

30. LEPIOTA CLYPEOLARIOIDES Rea.

In coniferous forest with some mixed deciduous trees, Lane County, October 24.

When the writer collected this beautiful species the solitary specimen was outstandingly distinct and easily identified. There was, however, some question concerning the exact color of the pileus of the European form but since then the determination has been confirmed by the Honorable Carleton Rea. The specimen conforms to Rea's description in every respect, and perhaps this constitutes the first report of the species from America.

31. **Armillaria badicephala** sp. nov.

Pileo 4–6 cm. lato, e convexo vel applanato, leviter umbonato; superficie sicca, parte centrali squamulosa badia vel "Sanford's brown," marginem versus pallide ochraceo-salmonia, radiatim squamis fibrillosis disco concolori; carne alba vel cremea, vulnerata ad "buff-pink" vel "onion-skin pink" tarde mutante, marginem sat tenui, centro crasso; sapore et odore leviter farinaceo; lamellis 6–7 mm. crassis, ex subaequalis semiellipticis vel semicirculis, albis ad "pinkish buff" tarde mutante, adnatis, remotiusculis, acie integris; stipite 5–7 cm. longo 8–12 mm. crasso, supra fibrillis arach-

noideis "pinkish buff," infra fibrillis striatis rufo-brunneis, cavo, intus albo vel "onion-skin pink; annulo arachnoideo, evanido; sporis late oblongis vel subglobosis, levibus, hyalinis, 5.5–7 × 5–6 μ.

Ad terram sub arboribus humilibus Coniferarum, prope Newport, Oregon, Amer. bor. Mensis Novembris.

Pileus 4–6 cm. broad, convex to plane, slightly umbonate; *surface* dry, squamulose on disk to fibrillose scaly toward margin, bay to Sanford's brown on the disk with scales toward margin concolorous on a background of light ochraceous-salmon; *flesh* rather thin at margin, thick on disk, white to creamy, slowly changing to buff-pink or onion-skin pink when exposed; *gills* 6–7 mm. broad, subequal or semi-elliptical to semi-circular in outline, white, changing to pinkish buff, adnate (sometimes sinuately-adnate) not crowded; *edges* even; *stem* 5–7 cm. long, 8–12 mm. in diam., striately-fibrillose scaly below the arachnoid, fugaceous; *ring*, arachnoid scaly above, scales reddish brown below, pinkish buff above, hollow, white to onion-skin pink within; *spores* short oblong to subglobose, 5.5–7 × 5–6 μ, hyaline; odor and taste slightly farinaceous.

Under low scrubby spruce trees, Newport. November. Collected by *Professor* and *Mrs. H. P. Barss.*

For those to whom the genus *Armillaria* is untenable this species comes closer to the *Tricholoma* concept than the other white-spored genera. It has several characters in common with *Tricholoma subpessundatum* Murrill but differs in surface of stem and cap, and the presence of an annulus.

32. ELASMOMYCES MATTIROLIANUS Cavara.

Under conifers, Yaquina John Point, South of Waldport, Lincoln County, May.

There was but one fructification found. This came just even with the surface of the forest floor duff, in which it was immersed. The duff here had been tramped down and was rather firm. The fungus showed as a white spot about a centimeter in diameter but when dug proved to be about 2 cm. in diam., and was creamy white. The stipe and sterile tissues are vesiculose-pseudoparenchymatous; *basidia* 2–4-spored; spores subglobose, echinulate, 1-vacuolate, dilute straw yellow, 7–11 μ.

This is perhaps the first report of this species in America.

33. PODAXIS PISTILLARIS (Pers.) Fries.

In sandy irrigated strawberry patch west of Bend on the McKenzie Pass highway, Deschutes County. June.

This plant was found in this very hot, semi-arid district. There was only one specimen. The strawberries had been recently cultivated and this fruiting body was next to a strawberry plant where the cultivator did not disturb it. This extends the northern limit of distribution for this genus in western United States.

34. MYCENASTRUM CORIUM (Guers.) Desv.

In volcanic ash soil, among sage brush, near Redmond, Deschutes County. June. Frequent.

35. CALVATIA SCULPTA (Harkn.) Lloyd.

In open woods under conifers at high elevations in Crater National Park, Mt. Hood, Mt. Jefferson, and the McKenzie Pass in the Cascade Mountains. Early summer.

This interesting puff ball has previously been reported from California. It might easily be put in another genus because of its striking peridial characters. It is similar in these characters to *Areolaria strobilina* (Kalchbr.) Forq. but differs in having smooth spores and no definite stipe.

36. LEUCOGASTER FOVEOLATUS (Harkn.) Zeller & Dodge.

In rocky but peaty soil. Breitenbush Lake, Jefferson County. August. First report from Oregon.

37. ALPOVA CINNAMOMEUS Dodge.

Among humus or moss in the forests of the Olympic Mountains, Washington, Corvallis, Benton County and Mt. Hood, Oregon, and Trinidad, Humboldt County, California. October.

It is of extreme interest that so shortly after Dr. Dodge described Alpova [4] from Michigan, we find that collectors from the University of Michigan have also taken specimens of this genus

[4] Dodge, C. W. *Alpova*, a new genus of Rhizopogonaceae, with further notes on *Leucogaster* and *Arcangeliella*. Ann. Missouri Bot. Gard. **18**: 457–464. *illus.* 1931.

in the States of Oregon and Washington. A note by Dr. C. H. Kauffman on collection No. 24 taken by L. E. Wehmeyer and Dr. Kauffman on Mt. Hood, Oregon, October 9, 1922, reads: "Among humus under log. Fruit body cream colored, then rufescent. Chambers at first filled with gelatinous substance; very gelatinous when mounted in water; becoming rufescent inside. Spores $5 \times 2\,\mu$."

A comparison of the western material with the syntype, No. Fp. 635, collected by A. H. Povah at Siskowet Outlet, Siskowet Bay, Isle Royle, Michigan, Sept. 4, 1930, leads to the conclusion that all should be incorporated in the one species, *A. cinnamomea*, To do this, however, some slight modifications of Dr. Dodge's description should doubtless be made. One of the Oregon specimens (No. 24) has a peridium $750\,\mu$ thick and the surface character is slightly different than described. In the Michigan material examined, as well as that from Oregon and Washington, often the peridium is much darker in the surface cells than farther inward, approaching a definite "rind" in appearance. The peridium is nevertheless always simple. The surface is very slightly pruinose.

The basidia are not always 8-spored. Five- to 11-spored basidia were definitely segregated both in the Michigan and western specimens. The spores are rarely $7\,\mu$ long, but mostly $4–5.3 \times 2–2.5\,\mu$.

Since the above note was prepared a collection was brought to the writer from Trinidad, California. There were more than 20 sporophores in this collection and Mr. H. E. Parks says a hundred could be found in one small area. This material from Trinidad had so much the general appearance of *Melanogaster* that I looked through my own unidentified collections referred temporarily to this genus and found one of *Alpova* taken in an oak thicket, near Corvallis, June 30, 1926.

Alpova is an interesting genus and provides one more example of the occurrence of species both on the Pacific slope and the Great Lakes-Central Canada region.

Specimens examined:

Michigan: Isle Royale in Lake Superior, Siskowet Outlet at Siskowet Bay, Sept. 4, 1930, *A. H. Povah*, Fp. 635, Syntype (in Herb. Univ. Michigan and Zeller Herb.).

Washington: Chehalis Co., Quiniault, Oct. 20, 1925, *C. A*

Brown and *C. H. Kauffman,* No. 3 (in Herb. Univ. Michigan and Zeller Herb.).

Oregon: Benton County, Corvallis, June 30, 1926, *S. M. Zeller* 7047 (in Zeller Herb.), Oct. 9, 1922, *L. E. Wehmeyer* and *C. H. Kauffman,* two collections, No. 7 and 24 (in Herb. Univ. Michigan and Zeller Herb.).

California: Humboldt County, Trinidad, August, 1933, *Wendall K. Parks* (*H. E. Parks,* No. 4620, in Zeller Herb., 8191).

IMPERFECTI

38. SPHAERONEMA PRUINOSUM Peck.

(Syn. *Glutinium macrosporum* Zeller, Jour. Agr. Res. **34**: 489–496. 1927.)

On apple bark, Hood River and Marian Counties. September to March.

About two years after I described *Glutinium macrosporum,* Dr. W. C. Moore, Harpenden, England, asked for material to compare with a fungus he had obtained from apple in England. Dr. Moore turned the two over to Miss E. M. Wakefield who found the English and American fungi to be distinct, but she also found that the writer had overlooked an older name for the fungus, namely, *Sphaeronema pruinosum* Peck. In the meantime through the courtesy of Dr. H. D. House I have been privileged to examine a part of Peck's type from Garrison, N. Y., and a collection by Dr. House from Newcomb, N. Y. I agree with Dr. Wakefield that *G. macrosporum* is synonymous with *S. pruinosum* but I believe the fungus is not a good species of *Sphaeronema.* The pycnidia, as illustrated in my paper (citation above), are strictly cylindrical, within and without and are not rostrate. I have not, however, had opportunity to study the types of *Glutinium* and *Sphaeronema.*

Neofuckelia Zeller & Goodding, gen. nov.

Stromate erumpenti, atro, discoideo vel pulvinato, subsessile vel stipitato; pycnidiis stromate omnino immersis, unistratosis, ostiolatis; poro rotundo, leniter papillato; sporophoris simplicibus vel breviter ramosis; sporulis hyalinis, continuis, fusiformibus vel bacillaribus.

Stroma erumpent, black, discoid to pulvinate, subsessile or stalked, with several locules (pycnidia) wholly immersed in the stroma to the same plane; *pycnidia* ostiolate; *ostiole* slightly papillate; *conidiophores* simple or branched; *conidia* hyaline, continuous, fusiform or bacillar.

This genus perhaps falls most naturally into the Phomaceae near *Dothiorella* because of the single stratum of pycnidia totally immersed in the black stroma. The latter, however, is usually stipitate in *Neofuckelia,* also a characteristic of the genus *Fuckelia.* In *Neofuckelia,* however, the pycnidia are ostiolate and in one stratum while in *Fuckelia* they are closed and merely locules without particular arrangement in a globose stroma.

39. **Neofuckelia pinicola** Zeller & Goodding, comb. nov.

(Syn. *Fuckelia pinicola* Zeller & Goodding, Phytopathology **20**: 563. *illus.* 1930.)

Stroma erumpent, black, scattered to gregarious, pulvinulate, subsessile to short-stipitate, 0.8–1.2 mm. diam., with one stratum of 16–35 locules (pycnidia) wholly immersed; *stromatic tissue* of pseudoparenchyma surrounded by a carbonous rind of the same structure; *stipe* of same structure, short (or sometimes almost wanting; *pycnidia* ovoid to subglobose, somewhat crowded, walls prosenchymatous, lining the stromatic parenchyma, 60–150 μ broad, 200–260 μ deep, ostiolate; *ostiole* slightly papillate, 25–32 μ in diam.; *conidiophores* produced from entire inner surface of pycnidia, hair-like, simple or branched; *conidia* hyaline, continuous, fusiform, narrowly ellipsoid, or bacillar, 8–11 $\times$ 1.7–3 μ.

Usually associated with *Atropellis pinicola* on cankered branches of living trees of *Pinus contorta, P. Lambertiana,* and *P. monticola.* Oregon, Washington, Idaho, and British Columbia.

One collection of stromata on stems of *Ribes Watsoniana* does not seem to differ from *N. pinicola.* This material was taken by *G. D. Darker* and *L. N. Goodding* east of Mt. Hood, Hood River County, Oregon, July 11, 1929.

40. DIPLODIA SYCINA Mont. var. SYCONOPHILA Sacc.

On honey fig (*Ficus Carica* var.) causing a die-back of the small twigs. Newberg, Yamhill County. January. Infrequent.

This is the first time this disease has been reported from Oregon.

41. BOTRYODIPLODIA CONGESTA (Lév.) Sacc.

On bark of *Juglans californica,* Corvallis. April.

The stubs where this fungus was plentiful on the bark had been grafted to the Persian walnut but the scions failed. The fungus may have caused the death of the bark on the stock. Several cases were observed.

42. **Melanconium candidum** (Peck) comb. nov.

Syn. *Melanconium bicolor* var. *candidum* Peck.

Acervuli discoid to broadly conic, black, erumpent, 1–1.5 mm. in diameter; *stroma* central, truncate-columnar, 240–320 μ broad at top, 400–800 μ broad across the discoid base, creamy-white, of hyaline prosenchyma; *locule* circinate-conic about the ectostroma, surmounted by the periderm; *conidiophores* from all walls of the locule, simple to branched, short, hyaline to brownish; conidia ovate, fuscus, smooth, 8–11 $\times$ 7 μ.

In bark of *Alnus rubra,* 20 miles south of Port Orford, Oregon, and Bainbridge Island, Washington. February to July.

This species was first described by Peck [5] as *Melanconium bicolor candidum,* but we believe it should be raised to specific rank for it is very distinct from *M. bicolor* Nees. The ectostroma is not so white as in the latter. It is creamy and the spores are 8–11 $\times$ 7 μ, while in *M. bicolor* they are 14–17 $\times$ 6–8 μ. This is distributed in Fungi Columbiani, 3334, as on *Alnus rubra* and the type in the N. Y. State Museum is on *A. rubra* but in Peck's description (l. c.) it is reported on " Bark of red mulberry, *Morus rubra* L."

43. GLOMERULARIA CORNI Peck.

Leaf-spot of *Cornus canadensis,* Sherwood Forest Camp, Mt. Hood Loop Highway, Hood River County, June. Collected by *Roderick Sprague* and *F. D. Bailey.*

Common in this type of mountainous location in the Northwest. To our knowledge not previously reported west of the Adirondack Mountains.

[5] New York State Mus. Bull. 150: 65. 1911.

44. RAMULARIA DESTRUCTANS Zinnsm.

Causing root-rot of *Panax quinquefolium*, Mayger, Columbia County. Infrequent. Late summer and fall.

45. RAMULARIA RUBICUNDA Bres.

On leaves of *Maianthemum bifolium* D. C. var. *kamtschaticum* (Gmel.) Jepson. Widely distributed in the Coast Range and westward. May to July. Very common.

This is a very severe leaf spot of this host. It has been observed from Curry County in Oregon to King County in Washington.

SPHAERIA ZEAE (DIPLODIA ZEAE) AND CONFUSED SPECIES

C. L. Shear and N. E. Stevens

(with 2 text figures)

Schweinitz' first use of the name Sphaeria zeae

Schweinitz (**16**) in 1822 described his first *Sphaeria Zeae*, as follows:

> "234 [*Sphaeria*] *Zeae* Sz.
>
> S. minuta aggregata conferta conica tuberculosa sulcata atra, intus succo repleta, villo albicante circumdata.
>
> Frequentissime provenit ad Zeae caulium emortuorum nodos vere et eos inquinat. Atra quasi rubescens. Siccata quasi spinulosa redditur."

In a manuscript of Schweinitz preserved at the Philadelphia Academy of Science, entitled, "Cryptogamarum praestantiorum Index novissime inventarum December 1820" is the following description of the same fungus:

> "× 204. *S. Zeidis* Nobis.
>
> S. minuta aggregata conferta et sparsa plerumque conica, tuberculosa, atterrima sed quasi rubescens-sulcata, sicco-intus fusco fructifero repleta-villo albido evanescente circum elata. Sicca tactu quasi spinulosa. Frequentissime ad nodos caulinum Zeidis provenit."

This is evidently a slightly modified form of the description published later and quoted above; the form of the specific name having been changed in the published work.

In another manscript of Schweinitz at the Philadelphia Academy, entitled, "Comentaria in Pyrenomycetum Friesii Species quae hic usque in American boreali obviae factae Sunt Ludovico Dav. de Schweinitz additis descripsionibus novarum 1828," is found the following record:

> "*D.* [*othidea*] *Zeae* L.v.S. Syn. Car. *Sph. Zeae* n. 234 non rara Carol. (nec. Perisp.) circum nodo caulinum zeae (caude distinguenda Cladospor. herbar. saepe in taliter aut aliter quasi caespitulatum indurescens) omnino haec pertinet."

Later (1832) Schweinitz (**17**) published a slightly modified description of the above form as follows, citing the original description of his No. 234 quoted above.

> "1866. 1. *D.* [*othidea*] *Zeae,* L.v.S., Syn. Car. no. 234, *Sphaeria,* frequens in Carolina nec in Pennsylvania obvia circum nodos caulis Zeae. Omnino hujus loci. Caule distinguenda a Cladosporio herbaceo indurato frequenter occurrens iisdem locis."

All the above descriptions of Schweinitz apply clearly to *Gibberella Saubineti* (Mont.) Sacc. and an examination of his specimens confirms this conclusion.

The original packet in Schweinitz' Herbarium at Philadelphia labelled "Sph. Zeae LvS. Salem" is empty, only a bit of cornstalk pith remaining glued to the paper.

Schweinitz' autograph specimen in Hooker's Herbarium at Kew which Schweinitz sent him in 1824 has been examined by the writers. It shows both ascospores and conidia of *Gibberella Saubineti.* This identification was first made in 1859 by Curry (**4**) in his report of a study of the same specimen (FIG. 2) which bears his notes. Curtis also examined Schweinitz' specimen of this species and has a marginal note in his copy of Schweinitz' North American Fungi opposite No. 1866, as follows: "Sph. saubineti M." In Michener's Herbarium, Mycological Collections, Bureau of Plant Industry, there is also a part of Schweinitz' original collection of this No. 1866 labeled "*Dothidea Zeae* Schw." This is also *Gibberella Saubineti.*

Schweinitz now having transferred his original *Sphaeria Zeae* to *Dothidea,* as indicated above, apparently considered the name eliminated from the genus *Sphaeria* and therefore free for application to another plant under the same genus.

SCHWEINITZ' SECOND USE OF THE NAME SPHAERIA ZEAE

In 1822 Schweinitz (**16**) also mentioned another *Sphaeria* on cornstalks, as follows:

"79. [S.] striaeformis α) frequens in Arundinaria macrosperma; β) in Juncis rarior; γ) in caulibus Zeae rarissima."

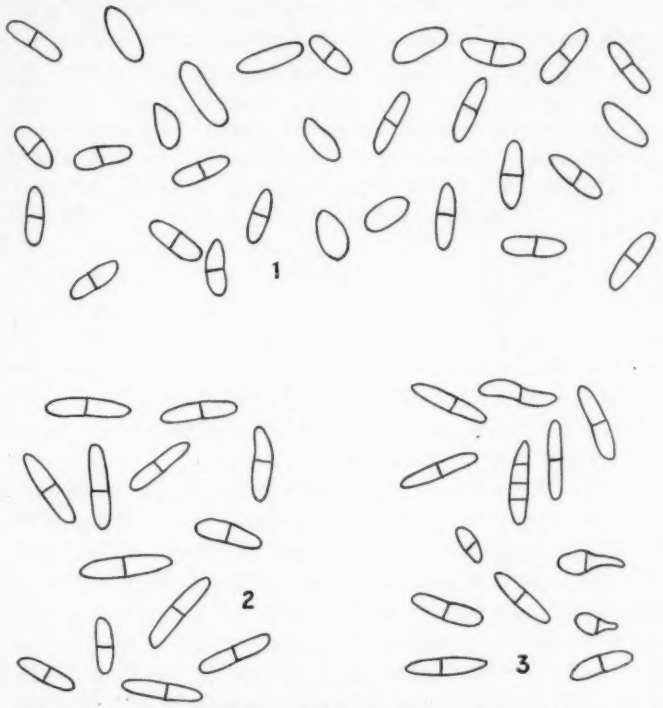


FIG. 1. 1, spores from one of Schweinitz' specimens of his second "*Sphaeria Zeae*" in the mounted collection at Philadelphia, apparently an aberrant form or a distinct species, × 400; 2, spores from Schweinitz' specimen of *Sphaeria Zeae* in Michener's herbarium in Washington regarded as the type, × 400; 3, spores from *Diplodia Zeae* (Schw.) Lév. on corn stalks from near Charlotte, North Carolina. 1934. × 400.

Later in Schweinitz' manuscript, "Comm. Pyren." etc. 1828, already referred to, this is described as follows:

> "273. S. Zeae LvS. Syn. Carol. n. 79 S. striaeformis, rara sed distinctissima in caulibus Zeae—S. epidermide elevato fuscotincta omnino nisi ostiolis tecta-seriatum disposita-brevis sed subconfluen. utrinq. acuminat. Perithecios in quoque caespitulo unis vel ternis subdistinctibus-ostiolis latis umbilicae

saepe unico intus prim. albofarctis dene cavis.
Nond. In Penyslv. observum."

In 1832 he (**17**) published a slightly modified form of the above description as a new species, his second *Sphaeria Zeae,* as follows:

"1451. 306. S. Zeae, L.v.S., Syn. Car. 79, rara sed bene distincta in caulibus Zeae, Salem et Bethl. S. omnino tecta, epidermide fusco tincta (ostiolis solis prominulis) satis elevata. Seriatim disposita, brevis, utrinque acuminata, subconfluens. Perithecciis binis vel ternis tantum in caespitulo, subdistantibus, primum albofarctis, demum evacuatis. Ostiolis latis, umbilicatis, saepe unico."

The original packet of this number in Schweinitz' herbarium labelled "Sphaeria Zeae L.v.S. Salem" is empty. Schweinitz himself sent some of it to Hooker (**18**). Later, Curtis took a part and finally Michener (**19**) divided the remainder, mounting part of it and keeping a bit for his own herbarium.

The following specimens referred by Schweinitz to his second *S. Zeae* have been examined by the writers:

There is a specimen in the mounted collection of Schweinitz' fungi at the Philadelphia Academy of Sciences, Philadelphia, showing the remains of a gummed paper strip which the writers (**18**) have shown to be found only on his earlier specimens, which were probably collected at Salem, N. C. There is also another specimen from Schweinitz which is apparently a part of the above, in Berkeley's herbarium at Kew marked "S. (seriata) Zeae L.v.S." and another part of the same specimen in the Curtis herbarium at Harvard. All of the above specimens bear pycnidia of a fungus resembling *Diplodia Zeae* of present authors. So far as can be determined from these scanty specimens the structure of the pycnidium is identical with the common form. The spores, however, differ from any other specimen thus far examined from any source, in the presence of numerous spores which are much wider in relation to the length and somewhat wider absolutely than those in typical *D. Zeae.* These wider spores are either one or two-celled. Figure 1 shows the range in shape and size of spores from these specimens. In size they range from 14.5–26 μ in length and from 3–9 μ in width. Having been unable to find any other specimen of this form in numerous collections on cornstalks from

North Carolina and elsewhere, we are at present unable to say whether this is a different species from the common *Diplodia Zeae* or an aberrant form of it.

While no fungus having a spore variation of this type has yet been seen by the writers, certain variations are very common in the ordinary *Diplodia* on corn. Spores with 2 or even 3 septa are not uncommon. One celled spores are found in some specimens and as already noted one cell of a spore is frequently smaller than the other. Figure 1, *B* shows the range of spore form in a single mount of what we regard as typical *D. Zeae*.

In Hooker's Herbarium at Kew there is also an autograph specimen of Schweinitz' labelled " Sp. cristata on *Zea* 1." Below this label in another hand is " *Diplodia Zeae* Lév. (*Doth. Zeae* Schw. Syn. U.S.)" and " *Sph. Zeae* Schw. Syn. U.S." On this specimen there are two fungi. Below the node on the stalk is typical *Diplodia Zeae* of recent authors. Above the node is a *Leptosphaeria* sp., to which Schweinitz apparently intended to apply the name *cristata*.

In the Michener Herbarium there is a Schweinitz specimen labelled " *Sph. Zeae* Schw. in *Zeae* Sal.-Beth." This bears good pycnidia of typical *Diplodia Zeae* of recent authors and is the specimen which we believe should be accepted as the type of *D. Zeae* (Schw.) Lév. as it agrees with the present general conception of this species, generally one septate, rather variable in length, but shows few irregularities in shape, except for the fact that one cell of the spore is frequently less well developed than the other. In size they are 24–29 × 5–6 μ (FIG. 1, *B*).

LATER STUDIES OF THESE FUNGI

Berkeley (2) in 1847 received specimens of a fungus on cornstalks from Ohio which he described as follows:

> " 139 *Sphaeria* (Seriatae) *Maydis*, n. sp.; maculis parvis subellipticis elevatis; peritheciis paucis; ostiolo unico conico, sporidiis oblongis curvulis uniseptatis.
>
> On dead culms of *Zea Mays*. Cincinnati, Ohio. May 1, 1841. T.G. Lea, Esq.
>
> Habit that of Sphaeria Arundinaceae. Spots minute, often purple-brown, punctiform, or subelliptic, rarely linear, con-

taining very few perithecia, with a single broad conical ostiolum. Sporidia oblong, slightly curved, uni-septate. Very different from Sphaeria Zeae, Schwein. ! as appears from an authentic specimen in Sir W. Hooker's Herbarium."

The *Sphaeria Zeae* Schw. referred to above by Berkeley was the first of Schweinitz' plants of that name which has been shown to be *Gibberella*. He apparently at that time had not studied Schweinitz' second *Sphaeria Zeae*. Berkeley's type which we have examined, is typical *Diplodia Zeae* of present authors.

In 1848 Léveillé (**11**) described Schweinitz' second *Sphaeria Zeae* as *Diplodia Zeae* as follows:

"*Diplodia Zeae*, n. sp. Conceptaculis gregariis innatis ovatis intus extusque nigris epidermide nigra tectis, ostiolis erumpen-acutis; sporis elliptico-elongatis rectis vel curvatis subopacis.-Hab. Tete de Buch prope Burdigalam ad culmos *Zeae Maydis*.

Sphaeria Zeae Schweinz. Syn. Fung. North Amer., p. 207—*Sphaeria dolosa* Pers. (herb. Lugd. Batav.)

Desc. conceptacles assez rapproches, globuleux au ovales, nichés dans l'épaisseur du chaume, et recouverts chacum par une portion d'épiderme noir, Ostioles aigus, proéminents à traverse la rupture de l'epiderme. Substance intérieure de couleur noire, composée de spores allongées, obtuses à une extrémité, aiguës à l'autram droites au courbées avec une colison médiane.

Obs. La ressemblance de ce *Diplodia* avec une sphérie cauli cole est frappant: mais quand on la soumet au microscope, l'illussion disparait: on voit alors qu'elle n'a de thèques, et que ses spores sont supportees par un clinode, dont les basides sont à peine visibles."

The above description leaves no doubt that Léveillé had the fungus to which this name is now applied in this country.

Currey (**4**) in working over the Sphaerias of Hooker's Herbarium at Kew examined Schweinitz' autograph specimen of his first *Sphaeria Zeae* (FIG. 2) and later (**5**) identified it as "nothing more than *Sphaeria pulicaris* Fr." (*Gibberella Saubineti*). He also examined Berkeley's type of *Sphaeria* [*Diplodia*] *Maydis* collected by Lea in Ohio and pointed out that it was entirely different from the *Sph.* [*Gibberella*] *Zeae* Schw.

Hazslinsky (**8**) in 1873 described and illustrated *Diplodia Zeae*

from specimens collected on cornstalks in Hungary and called it *Hendersonia Zeae* Currey.

Ellis and Everhart (7) in 1892, after examining a specimen of

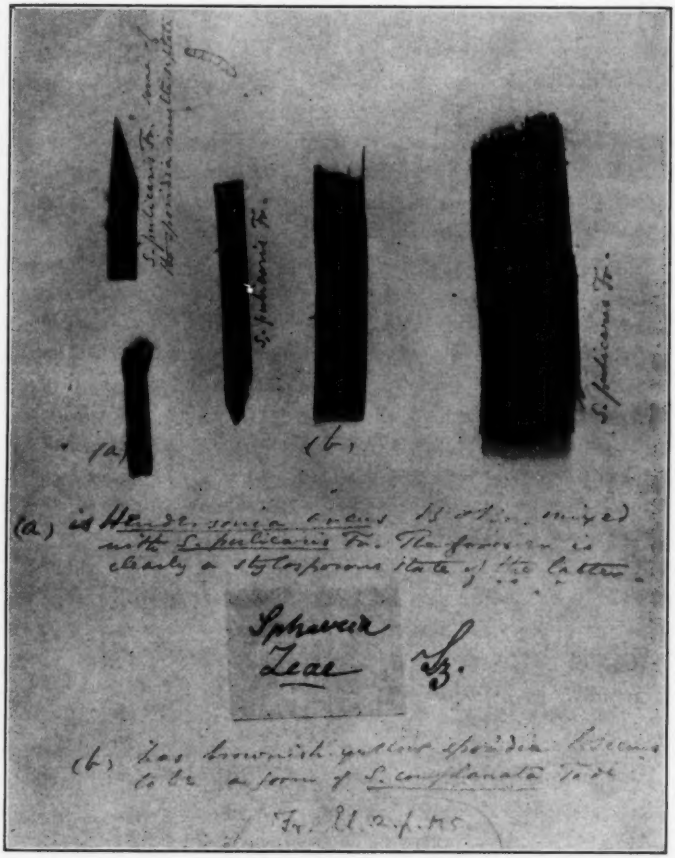

(a)
(b)
(a) is Hendersonia zeae (is mixed) with S. pulicaris Fr. The former is clearly a stylosporous state of the latter.
Sphaeria Zeae
(b) has brownish yellow spores & seems to be a form of S. complanata Fr.

FIG. 2. Schweinitz's autograph specimen of his first *Sphaeria Zeae* with Currey's notes.

Schweinitz No. 1451, his second *Sph. Zeae,* at the Philadelphia Academy say it "is the same as *Diplodia Zeae* Lév. in Ell. N.A. Fun. Exs. No. 31." This has been verified by an examination of

this number in the Mycological Collections of the Bureau of Plant Industry.

Under the name *Diplodia Zeae* (Schw.) specimens were distributed by Ravenel as No. *74* of his Fungi Caroliniani Exs. in 1852, and again in his Fungi Americani Exs. No. *393* in 1879. Ellis also distributed it in his N.A. Fun. Exs. No. *31* in 1878 as *Diplodia Zeae* Lév. and also in Ellis and Everhart Fun. Col. Exs. No. *73*, 1893, under the same name. The specimens of the above numbers in the Mycological Collections, Bureau of Plant Industry, have been examined and are all typical *Diplodia Zeae* (Schw.) Lév.

In 1888 Bennett (**1**) listed this fungus as "Dothiora zeae Sw." according to Burrill and Barrett (**3**).

In January 1909 Heald, Wilcox and Pool (**9**) described and illustrated this fungus and discussed its synonymy, deciding that the proper name was *Diplodia Zeae* (Schw.) Lév. There is no evidence, however, that they examined any type or authentic specimens of Schweinitz.

Burrill and Barrett (**3**) later in the same year discussed the synonymy, but also without examination of any type or authentic material. They assumed that all Schweinitz' specimens under the name *Sphaeria Zeae* referred to the same plant and they used the same name as Heald, Wilcox and Pool, *Diplodia Zeae* (Schw.) Lév.

In both the papers just mentioned, there are adequate illustrations of the pycnidia and pycnospores, those of the earlier paper being particularly good. The spore measurements as given by Heald, Wilcox and Pool (**9**) are 24–33 × 5 μ. Burrill and Barrett (**3**) noted a variation in the length of the pycnospores depending somewhat upon the culture medium used, giving the length as 22.1–28.5 and the width rather constantly at 5 and 5.2. These two papers were the first in which the pathological importance of this fungus was considered.

Saccardo (**15**), 1913, follows Heald, Wilcox and Pool, and adopts the name *Diplodia Zeae* (Schw.) Lév. for this fungus. He (**14**) had in 1884 used the synonym, *Diplodia Maydis* (Berk.) Sacc. for this species.

Spegazzini (**20**) in 1910 described "*Diplodia* (?) *maydicola*" as a new species. According to the original description this

agrees with *D. Zeae* (Schw.) Lév. Spore measurements are given as 24–26 × 5–7 μ, and though the writers have not seen Spegazzini's specimen they do not doubt its identity. Von Höhnel (**10**) in 1918 also expresses the same opinion in regard to Spegazzini's species.[1]

Woronichin (**22**) in 1922 proposed the new generic name, *Hendersoniopsis,* for this species. Later he found that name had been used by von Höhnel in 1918, and must be changed. In 1925 he (**23**) found the fungus on *Zea Mays* in Russia and changed the name to *Phaeostagonosporopsis.* None of his material has been seen but from his statement that it agrees with the descriptions and illustrations of Burrill and Barrett, and Heald, Wilcox and Pool, it is clear that his fungus is the same as ours.

Petrak and Sydow in Petrak (**12**) named the fungus *Macrodiplodia Zeae* (Schw.) P. & S. In 1926 the same authors (**13**) described the species in detail after having examined type and authentic specimens from Kew. The specimen labelled " S. (seriata) zeae " which was part of the early collection of Schweinitz' second *Sphaeria Zeae,* they regarded as a small spored form of *Diplodia Zeae* (Schw.) Lév. Their examination of the specimen labelled " *Sph. cristata* in Zeae " to which they refer as " *Sph. aristata* " gave the same result as we have indicated above.

While the fungus which is here regarded as *Diplodia Zeae* (Schw.) Lév. is not a typical *Diplodia* in the sense in which the present writers are using the name, that is, for the pycnidial forms of *Physalospora,* until its complete life history is known it does not seem desirable to transfer it to another genus as Petrak and Sydow, and Woronichin have done. The synonymy so far as known at present is as follows:

SYNONYMY

1822 *Sphaeria striaeformis* var. γ Schw.
1832 *Sphaeria Zeae* Schw.
1847 *Sphaeria Maydis* Berk.

[1] Since this was written Stevens, N. E. and Hoppe, P. E. (Plant Dis. Rep. **19**: 70, 1935) have obtained numerous cultures of *D. Zeae* from corn grown in Argentina, Uruguay and Mexico. The fungus probably occurs wherever *Zea Mays* is grown.

1848 *Diplodia Zeae* Lév.
1859 *Sphaeria* (*Hendersonia*) *Zeae* (Schw.) Curr.
1873 *Hendersonia Zeae* (Curr.) Hazsl.
1884 *Diplodia Maydis* (Berk.) Sacc.
1888 *Dothiora Zeae* (Schw.) Bennett.
1923 *Macrodiplodia Zeae* (Schw.) P. & S.
1925 *Phaeostagonosporopsis Zeae* (Schw.) Woronich.

Diplodia macrospora Earle (**6**) regarded by Petrak and Sydow as a variety of this species, while obviously closely related, has spores twice as long and seems to the writers deserving specific rank, as we have never found any intergrading forms.

From the facts above presented it is obvious that if the present International Code were followed literally, it would be necessary to apply the specific name *Zeae* Schw. to the fungus now generally known as *Gibberella Saubineti,* and the name *Maydis* Berk. to the fungus now nearly everywhere known as *Diplodia Zeae* (Schw.) Lév. The absurdity of such a procedure seems self-evident. Such changes could serve no useful purpose either scientific or otherwise and would only add to the confusion already too prevalent in mycological nomenclature. Since uniformity and stability in the use and application of names is the primary aim of nomenclature, *Diplodia Zeae* (Schw.) Lév. which has been the general and practically uninterrupted usage for over 50 years should continue to be the name applied to the fungus under consideration at least until its perfect stage, if any, is discovered.

LITERATURE CITED

1. **Bennett, Jas. L.** Plants of Rhode Island 87. 1888.
2. **Berkeley, M. J.** Decades of fungi. Lond. Jour. Bot. **6**: 326. 1847.
3. **Burrill, T. J. & Barrett, J. T.** Ear rots of corn. Bull. Exp. Sta Ill. **133**: 94–96. 1909.
4. **Currey, Frederick.** Synopsis of the fructification of the simple *Sphaeriae* of the Hookerian Herbarium. Trans. Linn. Soc. Lond. **22**: 330. *pl. 59, fig. 128.* 1859.
5. ——. Supplementary observations on the *Sphaeriae* of the Hookerian Herbarium. Trans. Linn. Soc. Lond. **25**: 259. 1865.
6. **Earle, F. S.** New species of fungi imperfecti from Alabama. Bull. Torrey Club **24**: 29–30. 1897.
7. **Ellis, J. B. & Everhart, B. M.** North American Pyrenomycetes, 745. 1892.

8. **Hazslinsky, F. A.** A Bánát-Erdélyi Határvidék gombaviránya. (The fungus flora of the frontier region Bánát—Transsylvania.) Math. Termész Közlem Bud. **10**: 48. 1872.
9. **Heald, F. D., Wilcox, E. M. & Pool, V. W.** The life-history and parasitism of *Diplodia Zeae* (Schw.) Lév. Ann. Rep. Neb. Agr. Exp. Sta. **22**: sep. 1–7. *pls. 10.* 1909.
10. **Höhnel, Franz von.** Fungi Imperfecti. Beiträge zur Kenntnis der Selben. Hedwigia **60**: 148. 1918.
11. **Léveillé, J. H.** Fragments mycologiques. Ann. Sci. Nat. III. **9**: 258. 1848.
12. **Petrak, F.** Mykologische Notizen. Ann. Myc. **21**: 189. 1923.
13. —— **& Sydow, H.** Die Gattungen der Pyrenomyzeten, Sphaeropsideen und Melanconieen. Rep. Spec. Nov. Reg. Veg. **42**: 1, 275–278. 1926.
14. **Saccardo, P. A.** Sylloge fungorum **3**: 373. 1884.
15. ——. Sylloge fungorum **22**: 1001. 1913.
16. **Schweinitz, L. D. de.** Synopsis fungorum Carolinae superioris. Edit. D. F. Schwaegrichen. Schr. Nat. Ges. Leipzig **1**: 48, 35. 1822.
17. ——. Synopsis fungorum in America boreali media degentium. Trans. Am. Phil. Soc. II. **4**: 207, 230. 1832.
18. **Shear, C. L., Stevens, N. E. & Tiller, R. J.** *Endothia parasitica* and related species. Bull. 380, U. S. Dep. Agr. 6. 1917.
19. —— **& Stevens, N. E.** Studies of the Schweinitz collections of fungi II. Mycologia **9**: 337. 1917.
20. **Spegazzini, C.** Mycetes Argentinenses. Anal. Mus. Nac. Buenos Aires **20**: 370. 1910.
21. **Sydow, H. & Sydow, P.** Beitrag zur Kenntnis Pilzflora der Philippinen-Inseln. Ann. Myc. **15**: 258. 1917.
22. **Woronichin, N. N.** Fungal and bacterial diseases of agricultural plants 255. 1922.
23. ——. A new parasite of maize in Transcaucasia. La Defense des Plantes **2**: 331–334. 1925.

BUREAU PLANT INDUSTRY,
WASHINGTON, D. C.

TETRACLADIUM MARCHALIANUM AND ITS RELATION TO ASTEROTHRIX, PHYCASTRUM, AND CERASTERIAS

JOHN S. KARLING

(WITH 5 TEXT FIGURES)

INTRODUCTION

In connection with studies to determine the susceptibility of species of *Isoetes* to *Cladochytrium replicatum* Karling a large, unusual aquatic hyphomycete was isolated from dead and decaying leaves of *I. lacustris* growing in the greenhouse tanks at Columbia. This fungus proved to be so unique and striking as to the size and shape of its conidia as well as their method of development that it was at first believed to represent a new genus and species of Fungi Imperfecti. Subsequent study of its development and morphology and a survey of the literature have shown, however, that it is undoubtedly identical with *Tetracladium Marchalianum,* which was discovered and established by De Wildeman in 1893. This saprophytic and monotypic genus was founded on material discovered among algae and the debris of higher plants in a pond in the Botanical Garden of Brussels, and since that time, because of its confusion with several genera of algae, has been the object of extensive discussion and controversy. De Wildeman noted at once the similarity of the conidia to the algae *Phycastrum longispinum* described by Perty in 1852 and *Cerasterias raphidioides* by Reinsch in 1867 and 1888, and expressed considerable doubt as to whether the organism in question was an alga or fungus. He submitted a sketch of this organism to Saccardo, who expressed the opinion that it was undoubtedly a fungus and closely related if not identical with his *Titaea callispora.* De Wildeman described it again in 1894 from stems of *Hippuris vulgaris,* classed it in the Phragmosporeae division of the family Mucedinaceae, and reported its further occurrence in France and

Switzerland. He again pointed out the striking resemblance of the conidia to the above mentioned algae, and particularly *Asterothrix microscopica* Kütz (1845–1849). In the following year Chodat, commenting on the forms which he had observed for several years in Geneva, maintained that *Tetracladium* and *Cerasterias* are identical and that the latter generic name should be excluded from the algae, on the grounds presumably that the organism to which it applied was not an alga but a fungus. In reply (**1895**) to Chodat's contention De Wildeman agreed that *Tetracladium Marchalianum* is synonymous with *Cerasterias raphidioides* var. *incrassatum* and *inaequale* as described by Reinsch in 1888 but quite different from the forms reported in 1867, and maintained that the genus *Cerasterias* should be retained in the algae for these earlier species.

Saccardo recognized De Wildeman's genus *Tetracladium* in 1899 and transferred it from the Phragmosporeae to the Staurosporeae division of the Mucedinaceae, next to *Titaea,* but in 1900 and 1907 Lindau referred it to the Phragmosporeae again, a grouping which has very recently been followed by Migula (**1934**). Clements and Shear (**1930**), on the other hand, place it in the Staurosporeae group of the family Moniliaceae.

In the meantime Printz (**1914**) revived again the questions of identity and synonymy of this organism. In material collected by Wille in Norway he found thalli of *Cerasterias raphidioides* which, from his drawing, appear identical with those of Reinsch of 1888, and he is convinced that this species is an alga, should be transferred to the genus *Asterothrix* Kütz, and is very similar to and possibly identical with *A. longispinum* or *A. tripus.* If the latter view is correct we would thus have a most confusing synonymy for *Tetracladium Marchalianum* De Wild., which would include indisputable algal species. In reply to Printz's claims De Wildeman (**1920**) called attention to this confusion and reasserted his previous contentions that *Cerasterias raphidioides* var. *incrassatum* and *inaequale* or *Tetracladium Marchalianum* is without question a fungus. In 1925, however, this species was reported again from Switzerland by Huber-Pestalozzia, who accepted Printz's synonymy and described it as *Asterothrix* (*Cerasterias*) *raphidioides* (Reinsch) Printz. Huber-Pestalozzia apparently never grew this

organism in culture, and his entire study is devoted to the variations and growth of the conidia or spores which he regards as individual thalli or plants. Although he figured and described these structures in considerable detail and believed he saw new thalli originating at their apices, he was still uncertain as to the systematic position and fungous nature of this species and expressed the opinion that the genus *Asterothrix* might represent a group of primitive fungus-like organisms.

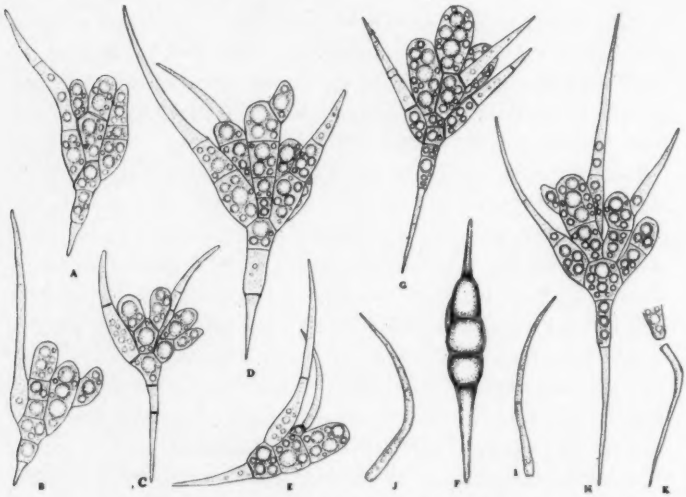


FIG. 1. Variations in the size and shape of conidia of *Tetracladium Marchalianum* De Wild.

Since that time *T. Marchalianum* has been reported from Canada by Lowe (**1927**) in a brief note entitled "*Cerasterias*, the Child of Sorrow of the Algologists." Spectrum analysis and culture work by Lowe proved conclusively the absence of chlorophyll in the spores and that the latter are true conidia which germinate readily on ordinary fungus media and produce well developed mycelial colonies within 48 hours. Unfortunately, Lowe gives no figures of the form he described. In the same year Printz (**1927**), apparently realizing the fungous nature of this species, excluded the entire genus *Cerasterias* from the algae and admitted the

synonymy of *C. raphidioides* and *T. Marchalianum*. However, he makes no distinction between *C. raphidioides* forms *tridens, tetradens, octodens,* and *obtusa* Reinsch of 1867 and *C. raphidioides* var. *inaequale* and *incrassatum* Reinsch of 1888, and excludes both of them as being fungi. In 1928, however, Collins listed the latter as an alga again and copied one of Reinsch's (**1888**) figures to illustrate the species as it occurs in North America. Except for Smith's (**1933**) statement that *C. raphidioides* var. *inaequale* and *incrassatum* is a fungus, subsequent reports of this species are lacking, as far as I am aware, and up to the present time it has been described from Germany, France, Belgium, Switzerland, Africa, Norway, Dutch Guiana, Canada, and the United States.

In view of the long controversy over the identity of *T. Marchalianum* and its confusion with the algal genera *Phycastrum, Polyedrium, Cerasterias,* and *Asterothrix,* the author feels that a brief description of the structure, development, and cultural characteristics of our form is highly worth while and necessary to demonstrate conclusively the fungous nature of this species and to clarify its position relative to the algae. Cytological studies on the structure of the mycelium and the development of the conidia are in progress and will be reported later.

STRUCTURE AND DEVELOPMENT OF THE CONIDIA, CONIDIOPHORES, AND MYCELIUM

As has been shown by De Wildeman, the conidia of *Tetracladium Marchalianum* are strikingly unique in shape and structure. They are hyaline, multicellular, flat, somewhat triangular in shape, and brush-like in appearance, with the basal or attachment cell serving as the handle. As to septation they are essentially phragmosporous, no dictyosporus conidia having so far been observed. Text figure 1 shows the range of variation in size and shape of conidia from corn meal agar cultures. The same range of difference has also been found in nature on decaying *Isoetes* leaves. As shown in this figure, the individual spores consist of from 8 to 18 cells extending out in a more or less flat plane at the apex of a central axis. Text figures 1*A*, 1*B*, 1*E*, and 1*F* show comparatively small conidia, while in the remaining figures they are more complex.

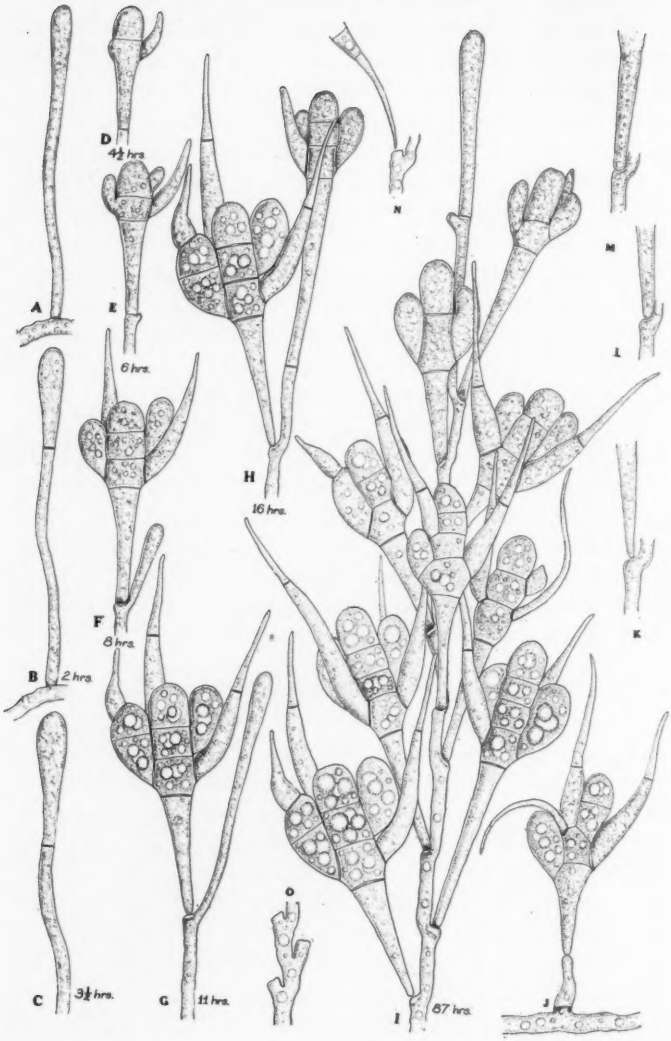


FIG. 2. Successive stages in the development of a conidiophore and conidia.

A conspicuous feature of all conidia is the presence of from one to four finely drawn out or needle-like cells, which extend considerably beyond the main body of the spore. The basal cell by which the conidia are attached to the conidiophores may also become greatly extended and pointed at maturity, so as to be indistinguishable from the more apical spines. All these needle-like extensions are very soft and pliable in spores which have just been formed, and may readily be bent and displaced by dissection needles. As the conidia grow old and become more or less dry, the spines may readily break off (FIG. 1*K*). In the majority of conidia one of the spines is usually more fine and stylus-like, and closely resembles the stylospores of *Phomopsis*. They generally break off from the conidia at maturity likewise, and in agar cultures may be found quite numerous in the vicinity of the spores. Figure 1*E* shows such a stylus-like spine attached to a conidium, while in 1*I* and 1*J* they are shown detached.

The other cells of the conidia are more or less globular in shape. At maturity they are generally filled with large and small refractive globules which may become so numerous as almost to obscure the cross septa in living material. Quite frequently they fill the cells completely and by mutual contact and pressure become more or less hexagonal in shape. Such globules may also be present in the bases of the spine cells, but it is not uncommon to find the latter completely hyaline. When viewed from the side, as in figure 1*F*, the conidia appear somewhat flattened, showing that the cells do not extend out in all planes. Due to their unusual shape and structure, it is of course difficult to give representative measurements of the spore. The large conidium shown in figure 1*H* measured 75 μ from tip to tip, and 22 μ at its broadest diameter; while those illustrated in figure 1*A* and 1*B* were 28 μ and 32 μ in length. De Wildeman found variations in length of from 10 to 108 μ.

The conidiophores are comparatively short and usually arise at right angles to the mycelium as somewhat club-shaped branches. The majority are single and unbranched, but numerous branched ones have been found both in agar cultures and on *Isoetes* leaves in nature. A series of successive stages of development of a conidiophore and conidia at different time intervals is shown in

figure 2 of material in a hanging drop chamber. The apical end of the conidiophore begins to enlarge (FIG. 2*A–2C*) and forms a cross wall which thus delimits the initial of the primary spore. Very shortly thereafter this initial may undergo further transverse septation (FIG. 2*D*, 2*E*) and develop buds or short branches which in turn are delimited by septa. At this stage the cytoplasm is quite dense and finely granular, and numerous small refractive globules begin to appear. These increase in size and number as the conidium develops, until at maturity, as noted before, they may be so large and numerous as to fill the cells entirely. By development of additional buds or branches and their growth and division in one plane (FIG. 2*E–2G*), the mature conidium is

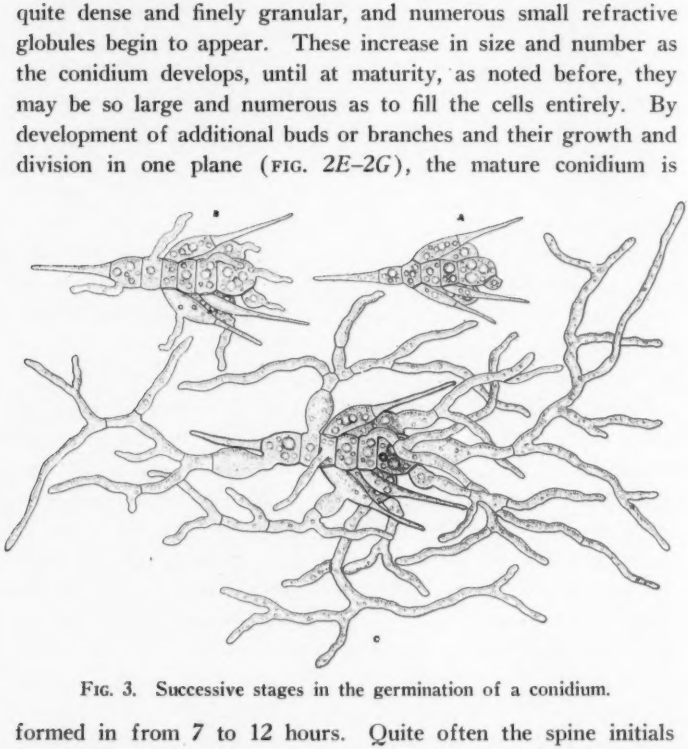


FIG. 3. Successive stages in the germination of a conidium.

formed in from 7 to 12 hours. Quite often the spine initials appear first (FIG. 2*D*) as more slender buds, but there seems to be no regular sequence in their appearance with respect to the other cells. At maturity they may or may not become septate and multicellular. It is obvious from this description that the multicellular condition of the conidia does not result from repeated divisions of a mother cell in a meristogenous manner, but fundamentally by the development, delimitation, and growth of buds or short branches.

In the meantime the conidiophore beneath has continued growth and formed the initial of the second conidium (FIG. *2E–2G*). The latter is very shortly delimited by a septum and develops in the same manner as the primary spore (FIG. *2H*); while the conidiophore continues on to form additional conidia in acropetal succession (FIG. *2I*). In agar cultures, as far as my observations go, it is rare to find more than five and six conidia on a single conidiophore. Two, three, and four are the usual numbers. Oftentimes they may be borne singly on short conidiophores, as is shown in figure *2J*. The conidiophore illustrated in figure *2I* is exceptional, according to my observations, and doubtless represents a maximum of complexity and development. In this figure are shown 9 conidia with the 10th in an incipient stage at the apex. As a result of this acropetal method of conidial development, the conidiophore may be somewhat irregular or slightly zigzag in outline with marked shoulders where the successive spores were borne. Quite frequently the shoulders may be so prominent (FIG. 20) as to look like short branches.

Particularly noteworthy are the changes which take place in the shape of the basal or attachment cell as the conidia mature. While attached to the conidiophore the basal end is rather blunt (FIG. *2M*), but as maturation continues it becomes finely drawn out (FIG. *2L, 2K, 2N*) and pointed like the tips of the apical spines. As a result, when the conidia are first observed separate from the conidiophore, it is difficult to determine where and how they are borne. In mature and old spores this basal spine may readily break off (FIG. *1K*), leaving the spore base blunt or rounded. This extension of the basal cell is not universal, however, since many conidia may be found in which it has undergone but little modification.

The conidia germinate readily in water and in standard culture media in 2 to 10 hours. Sometimes they may even germinate on the conidiophores before reaching maturity. All cells appear capable of germination, and it is not at all uncommon to find germ tubes growing out in all directions from the conidia. However, very few spores have been observed in which the tip cells of the spines were germinating. Successive stages of germination in a hanging drop culture are shown in figure 3. In the majority of

mature and old spores the cells tend to swell up and become somewhat vesicular before the germ tubes appear (FIG. 3*B*); and as a consequence the conidia increase considerably in size and volume. When the germ tubes first appear they may be fairly slender, but after attaining some length they increase in diameter, branch, and become septate. In a large number of conidia observed the first cell of the germ tube became greatly enlarged and vesicular like the cells of the spores (FIG. 3*C*). The latter, however, were still clearly distinguishable by their thick walls. In the process of germination the refractive globules may become broken up, and some of them wander out into the germ tubes. In other cases they may remain behind and gradually disappear, suggesting perhaps that they are reserve food which is used up in initial growth.

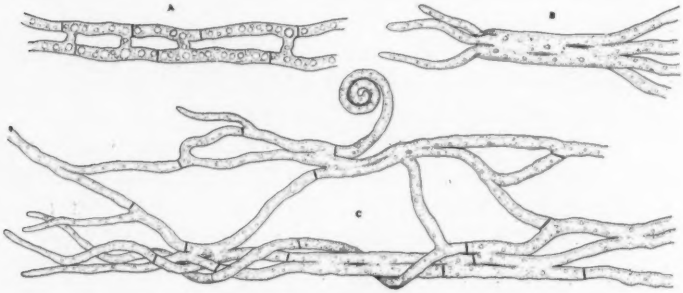


FIG. 4. Variations in hyphal anastomosis.

The mycelium is hyaline, septate, and highly branched and at maturity contains numerous refractive globules of various sizes like the conidia (FIG. 4*D*). In old cultures, particularly on potato dextrose agar, these bodies may often have a greenish yellow color, as Chodat has already described. This coloration together with the fact that the mycelial cells may sometimes break apart and appear as single individuals doubtless accounts partly for the inclusion of this fungus among the algae. Anastomosis is fairly frequent in hanging drop cultures, and is also common in nature and agar cultures. In figure 4 are shown some of the more unusual cases observed in hanging drops. It may be intercalary by the formation of short side branches (FIG. 4*A*), terminal (FIG. 4*B*), or lateral (FIG. 4*C*). In some hanging drop cultures anastomosis

has been so abundant as to form a network of filaments, and in other instances, an almost dendritic habit of growth. This may be partly due to the unusual conditions in the hanging drop chambers, since hyphal fusions have not been found to be so pronounced in nature. The diameter of the mycelium varies considerably and seems to depend to some extent on age and degree of anastomosis. Variations of from 3.5 μ to 10 μ have been observed in agar cultures.

CULTURAL CHARACTERISTICS AND PATHOGENICITY

Tetracladium Marchalianum has been grown on a large number of synthetic agars including corn meal, pH 6.0; potato dextrose, pH 5.5; Sab. dextrose, pH 5.7; prune, pH 5.7; Russell double sugar, pH 7.3; and Saccharosemannitol, pH 7.35. It grows slowly and well on all of these media, but fastest and best perhaps on corn meal and potato dextrose agars. On these latter two, colonies 7.5 cms. in diameter may be formed within a month. On cornmeal agar the colonies are symmetrically circular in outline and with well marked concentric rings, light pearl grey in color, smooth and comparatively thin; while on potato dextrose and Sabouraud's dextrose agars they are strikingly different: thicker, heavier, more felt-like with prominent concentric rings and conspicuous ridges and depressions radiating from the center. The surface of the colonies appears quite mealy, and the center is considerably elevated. In one-month-old colonies the central zone tends to be pinkish grey, then follows a mealy grey bank surrounded by a wider slightly lemon yellow zone, which finally fades out at the edge to a light pearl gray ring. These faint variations in color together with the mealy surface, radiating ridges, and depressions make a totally different picture from that presented on corn meal agar culture.

On saccharose-mannitol and Russell's double sugar agars with phenol red as indicators, growth is much slower; month-old colonies rarely being more than 2.5 cms. in diameter. They are generally heaped up or elevated in the center and markedly ridged. Acid production is quite pronounced and rapid, since within 12 to 18 hours the color of the agar has changed to amber yellow. On prune agar the colonies are again quite characteristic. The hyphae

appear to be more densely aggregated into strands, which radiate from the center and become markedly branched, frayed, fluffy, and lace-like at the edge. As a result the outline of the colony may become somewhat irregular. Concentric rings are none the less conspicuous, and the color of the colony as a whole is a dirty grey, becoming slightly lavender grey in older cultures. Growth is somewhat more rapid than on the double sugar agars; many month-old colonies being 3.5 cms. in diameter. Variations in habit and rate of growth naturally occur in all these agars, but the descriptions given above are fairly characteristic.

This fungus also grows well in liquid media. All of the above named agars were diluted to liquid consistency with distilled water, sterilized in flasks, and inoculated with small cubes of agar in which the mycelium only was growing. These cubes sank at once to the bottom, and within a fortnight vigorous hemispherical colonies had developed around them. The colonies continued to grow until the flasks were filled, and on reaching the surface spread out into a flat mat and formed abundant conidia. So far conidia have only been observed on the surface of such media.

As to the pathogenicity of the *Tetracladium Marchalianum* very little definite is known. De Wildeman, Chodat, Saccardo, Lindau, and Lowe describe it as a saprophyte, but Putteman, according to De Wildeman (**1920**) regards it as a dangerous parasite to certain vegetables, particularly leeks. Also Soraur has found it in Berlin on decaying hyacinth buds. Preliminary attempts have been made at Columbia to inoculate *Isoetes lacustris* in battery jars with rich spore suspensions, but all results have so far been negative. It has also been tested on detached fruits and vegetables such as oranges, apples, bananas, potatoes, etc., without positive results.

DISCUSSION

It is quite obvious from the above description that *Tetracladium Marchalianum* De Wild. is a fungus and should be clearly separated from any of the algae genera and species with which it has been related in the literature. While most mycologists have regarded it in this light, many algologists have confused it with old, doubtful, and poorly defined genera of the algae, with the result that the latter have acquired the stigma of uncertainty and

have been excluded from many textbooks of algology. Neither *Asterothrix, Phycastrum,* nor species of *Cerasteria* are included by Oltman (**1922**), Printz (**1927**), and Fritsch (**1935**), while Brunnthaler (**1915**), West (**1916, 1927**), and Smith recognize *Cerasteria* only in a limited sense. With the view of presenting this problem more concisely, I have brought together in figure 5 various illustrations of the questionable genera and species with which this fungus has been associated in the literature as well as the early figures of *T. Marchalianum* itself.

The genus *Asterothrix* with the species *microscopica* was established by Kützing in 1843, and as is shown in figure 5*A* the thallus consists of fairly short, branched filaments with oval and elongated cells or buds at the apices. In this latter respect it is strikingly like a monilioid fungus instead of an alga, which doubtless led Ström (**1923**) to the opinion that Kützing's figures relate to one of the lower fungi. Except for the habit of branching of the small thalli there is very little similarity, in the author's opinion, between this species and *T. Marchalianum.* On the other hand, *Asterothrix Pertyana* Naeg., as figured and described by Perty (*pl. 17, fig. 16*) is golden green in color and looks more like a blue-green alga, although the habit of branching is essentially the same as in *A. microscopica* Kütz. Rabenhorst (**1868**), however, regards *Asterothrix* as a doubtful algal genus and places the three species, *A. microscopica* Kütz., *A. tripus* Braun, and *A. Pertyana* Naeg. among the questionable forms. In 1887 Wolle included this genus in the Oscillarieae section of the Nostocaceae, and established an additional species, *A. Creginii,* from his American material. De Toni (**1907**) likewise classes *Asterothrix* among the blue-green algae but none the less regards it as a doubtful genus. In 1914 Printz recognized it as a valid genus of the Protococcaceae, but in 1927 he excluded it entirely from the green algae. Ström (**1923**), on the other hand, while including *A. microscopica* Kütz. among the Myxophyceae, is still doubtful of its algal nature, and believes it is closely related to some of the lower fungi. Finally, in 1925 Huber-Pestalozzia added two new species, *A. sessilis* and *A. spinulosa,* to the genus, but he is likewise doubtful as to its position and relationship among the algae.

Phycastrum longispinum, another species with which *T. Mar-*

chalianum has been associated in phycological literature, is shown in figures 5*B* and *C*. As figured by Perty it is a four-pointed unicellular organism with such deep lobing that no marked central axis or body is present. One of the spines is usually longer than the others (FIG. 5*B*), while another one drops off very early, leaving only three radiating spines as is shown in figure 5*C*. Perty figures this species as being deep green in color, which seems to leave little doubt as to its algal nature. In 1867 Reinsch transferred it to his newly created genus *Cerasterias* and changed the species name to *C. longispina*. In the following year Rabenhorst included it in *Polyedrium* and copied Perty's figures for illustration. Wolle likewise included it in this genus; but in 1888 Hansgirg transferred it to *Tetraëdron*, where it has been retained by many algologists, including Brunnthaler and West (**1927**). De Toni (**1889**), on the other hand, referred it back again to *Cerasterias*. Finally, in 1914 Printz placed it in the genus *Asterothrix* under the name *A. longispinum* and expressed the opinion that it was probably identical with *C. raphidioides* var. *inaequale* and *incrassatum* Reinsch (**1888**). In this manner *T. Marchalianum* came to be involved not only with *Cerasterias* and *Asterothrix* but with *Phycastrum* as well.

In figures 5*D*–5*G* is shown *Cerasterias raphidioides* Reinsch of 1867. Reinsch recognized four forms, *tridens, tetradens, octodens,* and *obtusa,* on the basis of the shape and number of spines present. Forms *tridens* and *tetradens* are essentially like *Phycastrum longispinum* Perty with the exception of the shorter and more blunt spines. Rabenhorst transferred this species likewise to *Polyedrium* and changed its name to *P. Reinschii,* and since that time has been placed in the genus *Tetraëdron* by Hansgirg, whose classification has been followed by Brunnthaler and West. There seems to be little doubt that *Phycastrum longispinum* Perty and *C. raphidioides* Reinsch of 1867 are unicellular algae and show no affinity with the fungi, but in *C. raphidioides* var. *inaequale* and *incrassatum* Reinsch of 1888 we are doubtless dealing with a species of an altogether different group. The outstanding difference, as De Wildeman points out, is the presence of rows of buds or cells in the axis of the spines (FIG. 5*H*–5*J*). Such structures are altogether lacking in the former species, according to the figures in

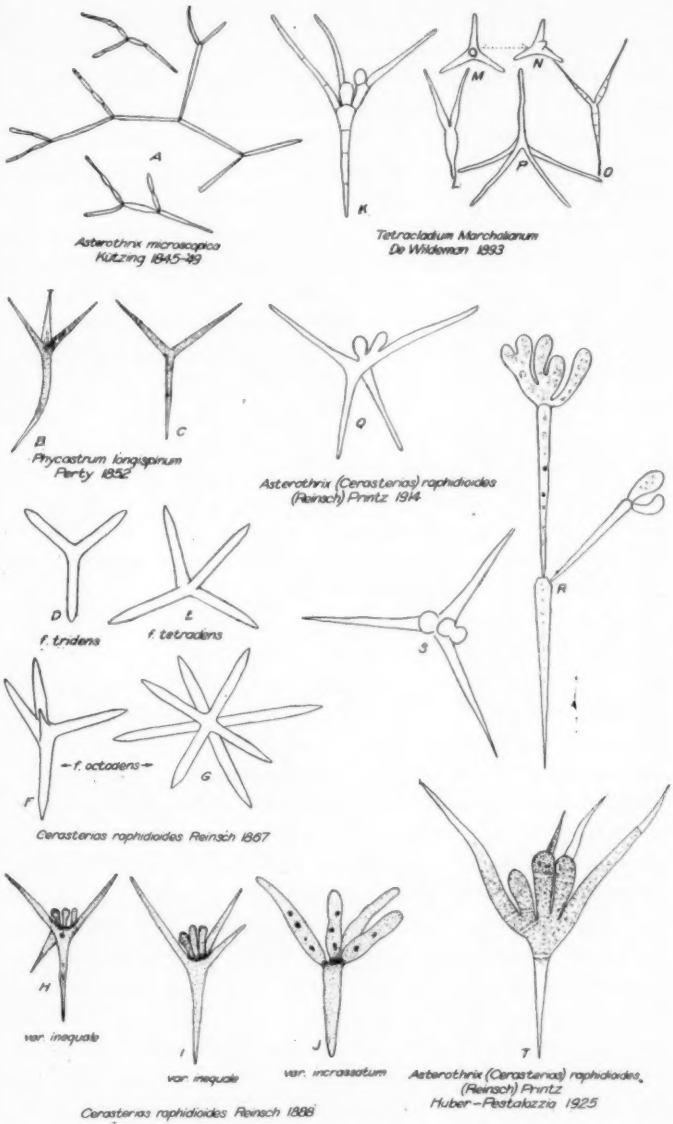


FIG. 5. Algal and fungus species with which *T. Marchalianum* has been confused.

the literature. To anyone who critically examines these and the other figures given by Reinsch and compares them with the conidia of *T. Marchalianum* it becomes at once obvious that vars. *inaequale* and *incrassatum* are identical and represent but variations of fungus spores found by De Wildeman. None the less, De Toni (**1889**) recognizes it as an alga and Collins (**1928**) copies the drawing shown in figure 5*H* as an illustration.

Tetracladium Marchalianum as figured by De Wildeman in 1893, is shown in figures 5*K–P*. A comparison of these figures and those of Perty and Reinsch (**1867**) leads one to suspect that in this first publication De Wildeman was dealing with a mixed culture. Figures 5*K* and 5*P* without question represent fungus spores, but the remaining ones are strikingly similar to the drawings of Perty and Reinsch (**1867**) and quite probably illustrate algae. It is doubtless the inclusion of such figures, it seems to me, that led algologists to believe De Wildeman was describing algal forms similar if not identical with Reinsch's (**1867**) *C. raphidioides*. His subsequent drawings are more distinctive of the fungus, but it seems as if the error which doomed *T. Marchalianum* to confusion had already been committed.

The only figure given by Printz in 1914 to illustrate his material of *Asterothrix* (*Cerasterias*) *raphidioides* is shown in figure 5*Q*. It is almost identical with figure 5*H*, with the exception that the reflexed spine is on the right side. This undoubtedly represents a fungus conidium, and Printz (**1927**) is clearly justified, in my opinion, in excluding it from the algae. However, his exclusion of *C. raphidioides* Reinsch of 1867 is obviously more open to question. Three of Huber-Pestalozzia's drawings of A. (*Cerasterias*) *raphidioides* (Reinsch) Printz are shown in figures 5*R–T*. These are so obviously conidia of *T. Marchalianum* that it is amazing how he could have any doubt as to their real identity. Figure 5*R* is particularly interesting in that Huber-Pestalozzia interprets it as the budding of a mother thallus to form daughter cells. To anyone who has grown and studied this fungus in culture, it is apparent that this figure really represents a conidiophore with two young conidia in the process of development. The fact that neither Huber-Pestallozia, Printz, nor Reinsch (**1888**) ever cultured this organism is doubtless the dominant factor in this

confusing situation. Had they done so, its identity as a fungus would have been immediately recognized.

In conclusion, these observations on *T. Marchalianum* confirm those of De Wildeman in practically every respect, with the exception of classification. While the conidia are multi-cellular and phragmosporous, their outstanding characteristic is one of shape. The spines are usually so elongated and divergent as to give the spores frequently the appearance of a three- to four-pointed star, and for this reason the author believes that this fungus should be retained in the Staurosporeae division of the family Mucidinaceae. Clements and Shear, as noted before, place *T. Marchalianum* in the family Moniliaceae on the basis of their inclusion of the Mucidinaceae with the former family.

As to the algae with which this fungus has been associated in phycological literature, the author agrees with De Wildeman, Smith, and West (**1916**) that the generic name *Cerasterias* should not be excluded from the algae but retained for such forms as *C. raphidioides* Reinsch 1867, *C. irregulare* Smith 1926, etc., either as a separate genus or a section of *Tetraëdron* as advocated by Hansgirg and Brunnthaler. *Cerasterias raphidioides* var. *inaequale* and *incrassatum* Reinsch (**1888**) are fungus conidia, and together with *Asterothrix* (*Cerasterias*) *raphidioides* (Reinsch) Printz 1914 are but synonyms of *T. Marchalianum* De Wild. 1893.

SUMMARY

1. *Tetracladium Marchalianum* De Wild. has been discovered on dead and decaying leaves of *Isoetes lacustris* growing in the greenhouse tanks at Columbia University.

2. It is without question a fungus which grows readily on ordinary culture media and produces well developed mycelial colonies.

3. The conidia are essentially three- to four-pointed, brush-like, flat, hyaline, multicellular, 22 μ to 75 μ in length, filled with refractive globules at maturity, and belong in the Staurosporeae division of the family Mucedinaceae.

4. The mycelium is hyaline, septate, highly branched, rich in refractive globules at maturity, and varies from 2 to 10 μ in diameter, depending somewhat on age and degree of anastomosis.

5. *Tetracladium Marchalianum* is doubtless an aquatic Saprophyte of wide occurrence. No evidence of parasitism has yet been observed on *Isoetes lacustris,* and it appears to develop only in dead and decaying tissues.

6. This fungus, in the author's opinion, is identical with *Cerasterias raphidioides* Reinsch (**1888**) var. *incrassatum* and *inaequale,* and consequently the name *T. Marchalianum* should supersede it, since it is confused with the algal genus bearing the same name.

DEPARTMENT OF BOTANY,
COLUMBIA UNIVERSITY,
NEW YORK CITY

LITERATURE CITED

Brunnthaler, J. 1915. Chlorophyceae. II. *In* A. Pascher's Die Sussw.-Flora Deutschlands, Osterreichs und der Schweiz **5**: 146.

Chodat, R. 1895. Materiaux pour servir a l'histoire des Protoccoidees. Bull. Herb. Boissier **3**: 109–114.

Clements, F. E. & C. L. Shear. 1931. The genera of fungi. New York.

Collins, F. S. 1928. Green algae of North America. New York.

De Toni, J. B. 1889. Sylloge algarum. **1**: 614–615.

——. 1907. Sylloge algarum. **5**: 682–683.

De Wildeman, E. 1893. Notes mycologiques. IV. Ann. Soc. Mic. Belge **17**: 35–68. *pl. 4–7.*

——. 1894. Notes mycologiques. VIII. Ann. Soc. Mic. Belge **18**: 135–161. *pl. 4–6.*

——. 1895. Notes mycologiques. XVI. Ann. Soc. Mic. Belge **19**: 191–232. *pl. 6–9.*

——. 1920. A propos du genre *Tetracladium.* Compt. Rend. Soc. Biol. Paris **83**: 192–194.

Fritsch, F. E. 1935. The structure and reproduction of the algae. Cambridge.

Huber-Pestalozzia, G. 1925. Zur Morphologie und Entwickelungsgeschichte von *Asterothrix* (*Cerasterias*) *raphidioides* (Reinsch) Printz. Hedwigia **65**: 169–178.

Kützing, F. T. 1845. Tabulae Phycologicae oder Abbildungen der Tange. Nordhausen.

Lindau, G. 1900. Fungi Imperfecti. Engler und Prantl, Nat. Pfl. I. 1^2: 448.

——. 1907. Fungi Imperfecti. Rabenhorst's Krypt.-Fl. 1^8: 409–410.

Lowe, C. W. 1927. *Cerasterias,* the child of sorrow of the algologist. Trans. Roy. Soc. Canada III. **21**: C 111.

Migula, W. 1934. Flora von Deutschland, Osterreich und der Schweiz. XI. 2.

Perty, M. 1852. Zur Kenntniss kleinster Lebensformen. Bern.

Printz, H. 1927. Chlorophyceae. *In* Engler, Nat. Pfl. 3.

——. 1914. Kristianialraktens Protococcoideen. Skr. Vidensk. Math.-Nat. Kristiana 1913, no. 6, 1–123. *pl. 1–7.*

Reinsch, P. F. 1867. Die Algenflora des mittleren Theiles von Franken. Nürnberg.

——. 1888. Familiae Polyedriearum Monographia. Notarisia **3**: 493–516. *pl. 4–7.*

Saccardo, P. A. 1899. Sylloge fungorum. **14**: 1067.

Smith, G. M. 1933. The freshwater algae of the United States. New York.

Ström, K. M. 1923. The alga-flora of the Sarek Mountains. Naturw. Untersuch. Sarekgebirges in Schwedisch-Lappland. **3**: 450.

West, G. S. 1916. Algae. Cambridge.

—— **& F. E. Fritsch.** 1927. A treatise on the British freshwater algae. Cambridge.

Wolle, F. 1887. Freshwater algae of the United States. Bethlehem, Pennsylvania.

THE TERMINOLOGY OF THE CRYPTOCOCCI WITH A NOTE ON CRYPTOCOCCUS MOLLIS [1]

RHODA W. BENHAM

(WITH 2 FIGURES)

The question of the correct terminology for those groups of fungi which resemble the yeasts, in that the vegetative body consists of round or oval budding cells, but differs from the true yeasts in not forming ascospores, has long troubled those working with these organisms. Many names have been suggested but none has been uniformly accepted. The two names which are most commonly employed are *Cryptococcus* and *Torula.* The latter is used most extensively by botanists and students of the industrial yeasts, the former by dermatologists.

The name *Torula* was first used for these yeast-like forms in 1838 by Turpin (**1**). Confusion resulted, however, as the name *Torula* had been given by Persoon in 1801 (**2a**, **b**) to one of the Fungi Imperfecti belonging to the Dematiaceae. Persoon's *Torula* is characterized by short dark hyphae with chains of globose to oblong conidia which readily fall apart. Nevertheless, Pasteur (**3**) and Hansen (**4**) in their later studies continued to use the name *Torula* for yeast-like forms without spore production, but having different intensities of alcoholic fermentation. In an attempt to remedy the confusion caused by the use of the name *Torula* for two quite different types of fungi, Berlese as quoted by Ciferri (**5**), in 1894 proposed to substitute for the name *Torula* Turpin (Pasteur-Hansen) the term *Torulopsis,* leaving the name *Torula* for Persoon's genus. This name was accepted by Saccardo (**6**) but it has never come into general use. Will (**7**) in 1916 distributed the non-spore forming, non mycelial ferments

[1] From the Laboratory for Medical Mycology and the Department of Dermatology, College of Physicians and Surgeons, Columbia University, New York, N. Y.

in two genera, *Torula* and *Eutorula*. Ciferri (**5**) in 1925 placed these forms in the two genera *Torulopsis* and *Eutorulopsis*, corresponding respectively to Will's *Torula* and *Eutorula*.

Clements and Shear (**8**) list the names *Torula* (Turpin), *Eutorula*, *Torulopsis*, *Eutorulopsis* and *Cryptococcus* as synonymous and give preference to *Torulopsis*.

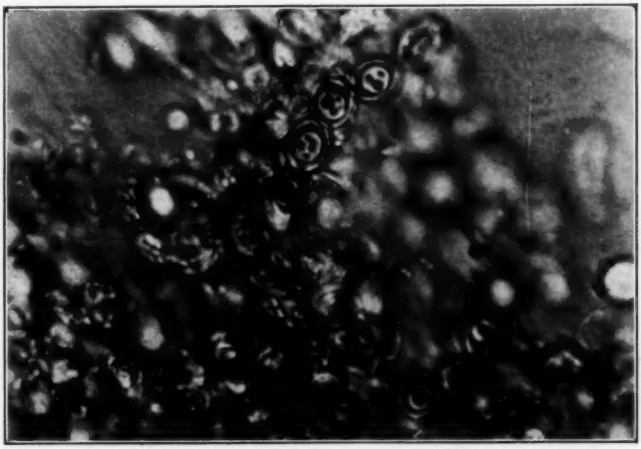


FIG. 1. Photomicrograph of *Cryptococcus mollis* from herbarium specimen, × 2000.

The name *Cryptococcus* was first used by Kützing (**9**) in 1833 for a microörganism which he found on moist window panes and which he classified among the algae. Vuillemin (**10**) in 1901 adopted the term for pathogenic yeast-like fungi which develop no ascospores. He gave the name *Cryptococcus hominis* to the fungus which Busse and Buschke had described as a *Saccharomyces* or "Hefe" without suggesting any specific name. Guilliermond (**11**) follows him in placing the pathogenic yeasts which do not sporulate in the genus *Cryptococcus* and similar non-pathogenic forms in the genus *Torula*, although he states they may all be considered part of the latter genus.

Ota (**12**) follows these authors in retaining the name *Cryptococcus* for non-mycelial, non-spore forming yeast-like fungi. He gives *Cryptococcus hominis* as an example of a pathogenic *Cryp-*

tococcus. Castellani (**13**) described a number of species of *Cryptococcus* and in his Adolph Gehrman lectures followed Vuillemin and Guilliermond in the use of *Cryptococcus* for the pathogenic and *Torula* for the non-pathogenic forms.

The majority of medical writers have followed this usage. On the other hand, Stoddard and Cutler (**14**) gave the name *Torula histolytica* to a fungus causing meningitis and this name has obtained wide acceptance although it seems certain (**15**) that their fungus is identical with that discovered by Busse and Buschke and previously named by Vuillemin *Cryptococcus hominis.* Recently the terms *Eutorula* and *Torulopsis* appear occasionally in descriptions of pathogenic forms. Castellani (**16**) refers to *Cryptococcus hominis* as *Torulopsis hominis* in a recent paper and Lodder (**17**) prefers *Torulopsis* as the generic name for this fungus.

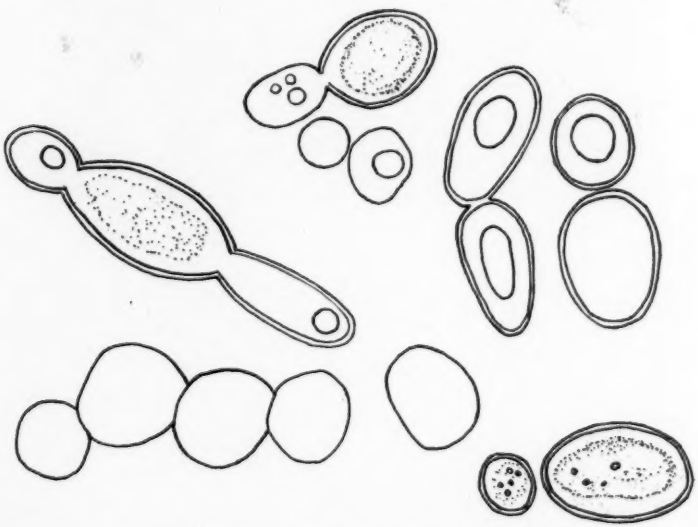


FIG. 2. Camera lucida drawing of Cryptococcus mollis from herbarium specimen, × 4000.

The use of so many different names for the same fungus has led to regrettable confusion, and a general acceptance of one term is desirable. Priority is the most secure ground for deciding on

the correct term but some authors would give consideration to common usage. Dr. C. L. Shear in a recent publication writes as follows: "Fortunately, thus far, no very widespread and serious attempts have been made to change our fungus names on a strict priority basis, and we know of nothing that would interfere more with the advancement and popularization of systematic mycology than a general attempt to apply this plan to the names of fungi, as it would result in a change of a great many of our best known names of genera and species. The names at present in general use should be conserved if we are ever to have a reasonably uniform and stable nomenclature." This idea seems especially important in medical mycology where many names which are nomenclatorially incorrect have become familiar by common usage.

In the present instance it should not be difficult to determine the correct name on grounds of priority. As has been stated, *Torula* (Persoon 1801) was a dematious fungus and does not belong to this group. *Cryptococcus* (Kützing 1833) takes precedence over *Torula* (Turpin 1838) and the other generic names discussed. The question would seem to depend then on whether the microorganism which Kützing described belongs to this group.

The objection has been raised that Kützing considered his *Cryptococcus* an alga. His description of the original species *C. mollis* is as follows: "Globuli mucosi hyalini non colorati microscopici in stratum indeterminatum mucosum facile secedens sine ordine aggregati. An feuchten und schmutzigen Fenstern." In his 1849 publication he lists thirteen species as follows: *C. nebulosus, natans, Rhei, Valerianae, mollis, inaequalis, roseus, carneus, coccineus, brunneus, vernicosus, Cerevisiae* and *Vini*. From the descriptions most of these species belong to the yeast-like fungi. None of them was a true alga. *Cryptococcus Fermentum* seems with certainty to belong to the yeast group and is generally accepted as a synonym of the comon yeast *S. Cerevisiae*. In his 1845 publication in Phycologia germanica, Kützing describes *C. Fermentum* as follows: "*C. Fermentum* (Hefe) Kugelchen 1/700–1/600‴ Gross, elliptisch, met 1–2 punkten in innern" and in the list of thirteen species, *C. Fermentum* is given as a synonym for *C. Cerevisiae*. Saccardo regarded the term *Cryptococcus* as

a synonym of *Saccharomyces*. The name is not used by algologists.

In Kützing's 1833 publication, Decas III, is to be found together with the description of the species *Cryptococcus mollis*, an herbarium specimen. This publication is to be found at The New York Botanical Garden. Through the kindness of Dr. J. H. Barnhart and Dr. M. A. Howe, I have been able to examine some of this material.

A little of the dried specimen was scraped off in water and later mounted in glycerine for study. The material is very well preserved and one can note a number of organisms present, including some bacteria. There is nothing in the material that would suggest an alga. By far the most abundant organism present is in the form of oval cells which resemble the yeasts. There is definite evidence of budding as may be seen in figures 1 and 2, though this was not mentioned in Kützing's description. The buds often remain attached forming chains of cells, but nothing like a true mycelium is seen. This form then resembles very much the *Cryptococcus* forms, and it seems clear that the name was given to yeast-like fungi. Although it would be impossible to identify *Cryptococcus mollis* with certainty, it seems evident that it was not an alga but a yeast-like fungus similar to the forms which have since been called *Cryptococcus, Torula, Eutorula* and *Torulopsis*. This would seem to establish the priority of Kützing's term.

So much for priority. Usage is less easy to determine, but the term *Cryptococcus* seems at least as familiar as *Torula* and is more commonly employed by medical mycologists than the latter term. The names *Torulopsis* and *Eutorula* have no wide currency. It would seem then that the generic name *Cryptococcus* deserves preference on grounds both of usage and of priority.

In conclusion it is suggested to emend Vuillemin's definition of the genus *Cryptococcus* somewhat as follows:

Order Moniliales: Family Pseudo-saccharomycetaceae, genus *Cryptococcus*.

Unicellular fungi consisting of round or ovoid cells occasionally in chains but never forming a well-defined mycelium. Repro-

duction by one or more buds; no ascospores. Growth on artificial media in pasty or dry colonies, white or colored.

It is further recommended that the generic names *Torula, Torulopsis,* and *Eutorula* be discarded as being synonymous with *Cryptococcus.*

In view of the fact that the original species *Cryptococcus mollis* is of such doubtful identity we would suggest *Cryptococcus hominis* Vuillemin as a representative species. It may be defined as follows:

Cells round to oval 3–8 μ in diameter, usually 4–5 μ. Contents granular with lipoid globules, wall distinct and surrounded by a clear zone of capsular substance. Buds usually single. In tissues of host occur scattered through a clear homogeneous substance forming a zoöglia-like mass. Giant colonies circular with a smooth glistening surface and mucoid consistency, color white or tan, or deep brown. Frequently pathogenic for man, rats and other animals. Habitat: Tissues of infected humans, horse and cheetah, skin and intestinal canal of normal humans.

LITERATURE CITED

1. **Turpin, P. J. F.** Sur la cause et les effets de la fermentation alcoolique et acéteuse. Compt. Rend. Acad. Paris **7**: 369–402. 1838.
2. **Persoon, C. H.** (a) Synopsis methodica fungorum. Goettingae, 1801. (b) Mycologia europaea. Erlangae, 1822.
3. **Pasteur, L.** Études sur la bière, ses maladies causes qui les provoquent, procédé pour la rendre inaltérable, avec une theorie nouvelle de la fermentation. Paris (Gauthier-Villars), 1876. (Page 387.)
4. **Hansen, E. C.** Sur les "Torulas" de M. Pasteur. Medd. Lab. Carlsb. 2^2: —. 1888.
5. **Ciferri, R. & Redaelli, P.** Studies on the Torulopsidaceae. Ann. Myc. **27**: 243–295. 1929.
6. **Saccardo, P. A. & Saccardo, D.** Sylloge fungorum omnium hucusque cognitorum. **18**: (Supplementum universale Pars VII) Patavi 1906. (Page 495.)
7. **Will, H.** Beiträge zur Kenntnis der Sprosspilze ohne Sporenbildung. VI. Mitteilung. Die Torulaceen. Centralb. Bakt. **46**: 226–281. 1916.
8. **Clements, F. E. & Shear, C. L.** The genera of fungi. New York (Wilson), 1931.
9. **Kützing, F.** (a) Algarum aquae dulcis Germaniae Decas III. 1833. (b) Phycologia germanica. 1845. (c) Species algarum. 1849.

10. **Vuillemin, P.** Les Blastomycétes pathogènes. Rev. Gén. Sci. **12**: 732–751. 1901.
11. **Guilliermond, A.** The yeasts. New York (Wiley), 1920.
12. **Ota, M.** Beiträge zur Morphologie, Biologie und Systematik des pathogenen, asporogenen Sprosspilze. Dermat. Woch.-Schr. **78**: 216. 1924.
13. **Castellani, A.** Fungi and fungous diseases. Arch. Derm. & Syph. **16**: 383. 1927.
14. **Stoddard, J. L. & Cutler, E. C.** *Torula* infection in man. Monogr. Rockefeller Inst. Med. Res. **6**: 1–98. 1916.
15. **Benham, R. W.** The fungi of blastomycosis and coccidioidal granuloma. Arch. Derm. & Syph. **30**: 385. 1934.
16. **Castellani, A.** Observations on fungi isolated from cases of blastomycosis pulmonalis in North America and Europe. Remarks on Blastomycetin. Jour. Trop. Med. **36**: 297–322. 1935.
17. **Lodder, J.** Die anaskosporogenen Hefen. Amsterdam, 1934.

CYTOLOGICAL STUDIES OF THE TREMELLACEAE[1] III. SEBACINA[2]

R. M. WHELDEN

(WITH 3 TEXT FIGURES)

The genus *Sebacina* comprises small usually very inconspicuous fungi apparently wide spread in occurrence, particularly in North America. While it has been split into several genera, Rogers (**4**) has pointed out that such a separation is unnecessary and has reduced the segregates to subgeneric rank. The writer has followed him in this, considering all resupinate tremellaceous fungi with waxy consistency to be species of *Sebacina*.

Three species were selected for intensive study; these were *Sebacina deminuta* Bourd. (*Bourdotia deminuta* Bourd.), *S. fugacissima* Bourd. & Galz.,[3] and *S. epigaea* (Berk. & Br.) Bourd. & Galz. The first species forms extremely thin greyish patches on the bark and wood of a partially decorticated fallen poplar trunk. On drying, these patches shrink to a faint inconspicuous glair easily overlooked. This species is characterised by having in the hymenial layer elongate hyphal tips, the gloeocystidia, whose protoplasm contains much yellowish " resinous " substance. *S. fugacissima* has brownish fruit-bodies somewhat more conspicuous than those of *S. deminuta*. In these fruit-bodies occur cystidia, enlarged hyphal tips devoid of protoplasmic content, projecting up through the hymenium. The third species studied, *S. epigaea*, has much larger light brown fruit-bodies growing over the ground,

[1] Contribution No. 134 from the Laboratories of Cryptogamic Botany at Harvard University.

[2] The writer is pleased to acknowledge his gratitude to Dr. D. H. Linder, who collected and preserved in the field much of the material used in the present study. The writer also wishes to thank Prof. W. H. Weston, Jr., for his helpful criticisms during the writing of this paper.

[3] Specimens of this fungus were submitted to Prof. G. W. Martin, who very kindly determined it as *S. fugacissima* Bourd. and Galz.; for this determination I am indeed grateful.

fallen leaves and debris. In these fruit-bodies neither cystidia nor gloeocystidia were present at any time during development.

HISTORICAL BACKGROUND

Comparatively little work has been done on the cytology of this group. In 1902 Maire (**2**) briefly described the development of the basidia of *S. effusa* Bref. In the young basidium the two nuclei with densely staining nucleoli and rather small karyosomes united to form the rapidly enlarging fusion nucleus in which the karyosomes were "en forme de longs filaments chromatiques, très fins, pelotonnés lâchement." When the basidium was fully mature there appeared extending between definite centrosomes a transversely oriented spindle at the center of which was a chromatin mass which sparated into two groups each having two chromosomes. Subsequently the two daughter nuclei, separated by a longitudinal septum, divided again in a way very much like the first division, after which each nucleus migrated into a spore. On germination of this spore another nuclear division might occur.

In 1924, Neuhoff (**3**) verified in the main Maire's work, although he could not determine the number of chromosomes, due to their minute size. Neuhoff also described, in *S. calcea* (Pers.) Bres., the history of the fusion nucleus, in which he saw two faintly staining chromatin nets and a tiny black-staining dot giving place to a long persisting spireme. When this broke up into chromosomes, apparently four in number, the nuclear membrane, previously distinct, became vague, while the transversely oriented spindle formed. This first nuclear division was followed immediately by a second, after which there occurred a considerable increase in the volume of the hypobasidium. Only when this increase was complete did the usually obliquely directed septa divide the hypobasidium into four uninucleate segments.

Kühner, in 1926 (**1**), noted in the fusion nucleus in *S. gloeocystidiata* Kühner the presence of fine regular chromatin threads and a distinct nucleolus. As division progressed, the nucleolus disappeared while the chromatin threads contracted and became irregular, finally giving rise to an indistinct number of chromosomes massed at the center of the transversely oriented spindle. A second division followed the first, so that eventually there were

formed four nuclei each of which entered a spore. Kühner alone seems to have noted the presence in the hymenium of any other object than basidia. He described the gloeocystidia as elongate bodies densely packed with golden-yellow drops of a highly refractive substance. These gloeocystidia, in which he saw no nucleus, had helical bands of some substance, which he considered to be vestiges of cytoplasm.

MATERIAL AND METHODS

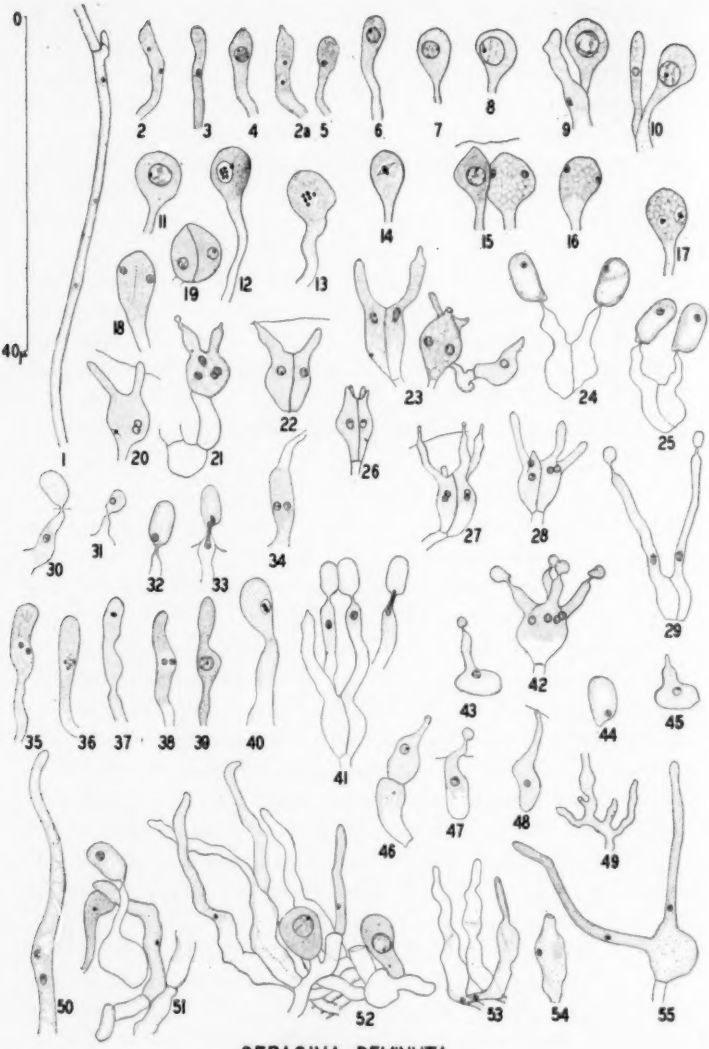
As described in an earlier paper of this series (**5**), each collection of specimens was divided into two or more portions, each portion was killed in the field by one or the other of the different fixing fluids and subsequently was stained by one of several different methods. As in the genera *Tremella* and *Exidia*, Haidenhain's iron-alum-haematoxylin gave by far the most satisfactory nuclear figures.

DESCRIPTION

The same general structure is revealed in sections cut through the center of the fruit-bodies of all species of *Sebacina*. Over the surface of the substratum there spreads a layer of closely interwoven hyphae, which in *S. fugacissima* is so dense as to render difficult the determination of individual hyphae. Above this basal layer arises a much looser stratum of hyphae extending into the superficial hymenium. In *S. deminuta* the subhymenial portion of the fruit-body is rarely more than 20 μ thick, in *S. fugacissima* it ranges up to 100 μ, while in *S. epigaea* the thickness is from 600 to 700 μ. Dried material brought into the laboratory and wetted seldom yields measurements equal to those obtained in the field.

In all species here studied the short segmented hyphae in the lower region seemingly are nearly devoid of protoplasm, while the more loosely arranged hyphae of the upper layer have longer, usually binucleate segments, filled with protoplasm (FIG. 1: 1). Near the surface these frequently end in long multinucleate tips, particularly in *S. epigaea* (FIG. 3: 25–28). No clamp connections nor interhyphal fusions were seen in any part of the fruit-bodies.

In the hyphal tips, often much branched, nuclear divisions are frequently observed in abundance. The small size of the nuclei, from 0.5 to 1 μ in diameter, makes it very difficult to determine



SEBAGINA DEMINUTA

FIG. 1.

the number of chromosomes. Three chromosomes are always seen; frequently there is observable near the chromosomes a minute object, which under certain conditions gives an impression of four chromosomes in the nucleus (FIG. 3: 28, lowest nucleus).

The youngest basidium initials, always binucleate, differ from the hyphal tips in having slightly denser protoplasmic contents, and a somewhat greater diameter (FIG. 1: 2; FIG. 2: 1, 2; FIG. 3: 1, 2). Even before the two small nuclei fuse, enlargement of the hypobasidium is quite noticeable, particularly in *S. deminuta* (FIG. 1: 3, 5). This enlargement becomes much more pronounced subsequent to the fusion of the nuclei and continues until the fully mature hypobasidium obtains (FIG. 1: 4, 6–7; FIG. 2: 3–9; FIG. 3: 3–7). The dimensions of the latter are extremely variable, but average as follows: in *S. deminuta,* they are subspherical bodies about 5 to 6.5 μ in diameter (FIG. 1: 8–11); in *S. fugacissima,* slightly larger bodies averaging $8 \times 6.3\ \mu$ (FIG. 2: 10); in *S. epigaea* much larger and often more irregular objects varying from $10 \times 7\ \mu$ to $15 \times 8\ \mu$ or even larger (FIG. 3: 8, 9).

During this enlargement of the hypobasidium, characteristic changes occur in the fusion nucleus which has increased in size correspondingly. In the species of *Sebacina* here studied fusion of the nucleoli is often delayed until considerable enlargement of the fusion nucleus has occurred (FIG. 1: 4). From the beginning of this enlargement the chromatin has been aggregated into definite linear masses which are located near the surface of the nucleus. While these masses are not as distinct as are those in the fusion nuclei of *Tremella* or *Exidia,* the number seems quite definitely to be six (FIG. 2: 11), a fact borne out when contraction of the masses has given rise to the six very small chromosomes. It is interesting to note that, particularly in *S. epigaea,* there appears to be irregularity in the time of contraction of these prochromosomes, so that chromosome-like bodies appear on the surface of the very large fusion nucleus (diam. 5 μ) while most of the chromatin remains in the very long patches (FIG. 3: 9). In this species also the number of chromosomes finally formed is difficult to determine, due to the tendency of the chromosomes to clump, and to the apparent fact that the individual chromosomes are unequal in size. Favorable cases indicate the number to be six (FIG. 1: 12, 13; FIG.

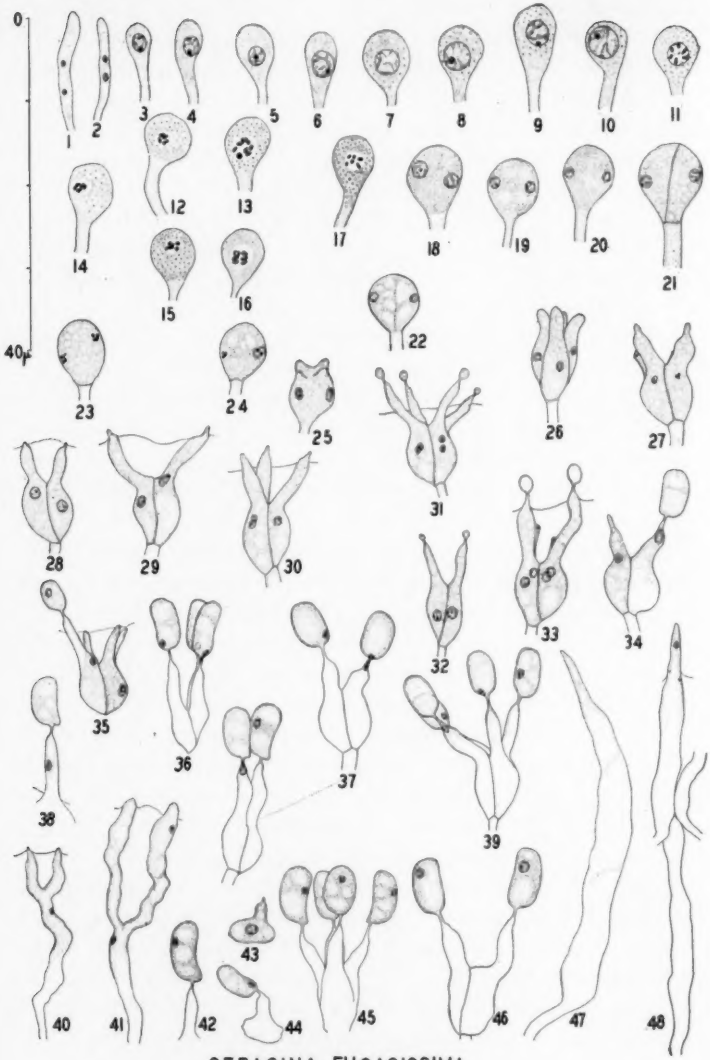

0
40
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
SEBAGINA FUGACISSIMA

FIG. 2.

2: 12–17; FIG. 3: 11–13). This conclusion is supported by observation of dividing nuclei in the actively growing vegetative hyphae of young fruit-bodies (FIG. 3: 25–29); which show the haploid number as three.

In the fusion nucleus, the chromosomes lie clumped quite closely on the very vague transverse spindle (FIG. 1: 4); when the chromosomes are formed, both nucleolus and nuclear membrane have disappeared. Although at each pole of the spindle, a suggestion of a dark staining object appears, no definite centrosomes can be distinguished. The two daughter nuclei, quite variable in size in each of the three species, are eventually formed far apart near the wall of the hypobasidium, in which they are usually situated at about the same level (FIG. 1: 15, 16; FIG. 2: 18–20; FIG. 3: 14). The second nuclear division, of one or both nuclei, occurs immediately after the first, resulting usually in four nuclei from 0.5–1 μ in diameter, that lie near the lateral surface of the hypobasidium (FIG. 1: 17, 20; FIG. 2: 23–25; FIG. 3: 15).

In general, the time of septum formation is not correlated with the divisions of the nucleus. At the time of the first, and often the second of the nuclear divisions, the basal septum cuts off the hypobasidium, frequently several μ below the enlarged portion, notably in *S. epigaea* (FIG. 1: 18; FIG. 2: 22; FIG. 3: 11–15). The longitudinal septa, frequently directed obliquely in the hypobasidium, are formed soon after the nuclear divisions are completed (FIG. 1: 18, 19; FIG. 2: 21, 22). The cytoplasm of the mature hypobasidium, which is commonly either two or four celled, very rarely three-celled, has become increasingly vacuolate.

Only rarely do the epibasidia begin to form before the nuclear divisions are completed. In young fruit-bodies of *S. deminuta*, the epibasidia, which first appear as minute bulges from the apical surface of the hypobasidium, grow rapidly to short, irregular, somewhat obconical structures, generally spreading at a considerable angle one from the other (FIG. 1: 22). The tip of each epibasidium gradually narrows to form a slender sterigma about 1 μ long at the end of which the spore develops (FIG. 1: 21, 22, 27). In the older fruit-bodies the much longer epibasidia grow directly out to the surface of the "jelly" (FIG. 1: 29, 41). In *S. fugacissima*, the very coarse epibasidia, usually from 2 to 2.6 μ in diam-

eter, grow outward in rather irregular fashion to a length of 6 to 8 μ then abruptly narrow to the very slender sterigmata (FIG. 2: 26–30). In *S. epigaea,* the epibasidia, strikingly unlike those of the other two species here described, first appear as coarse tubes, from 2.0 to 2.5 μ in diameter, arising in a compact group from the apex of the hypobasidium (FIG. 3: 16). As the epibasidia grow to their maximum length of 50 to 80 μ, the diameter gradually increases to 3 to 3.5 μ. Throughout this growth the two, or more frequently four, epibasidia are nearly parallel (FIG. 3: 22–24, 31). On reaching the " jelly " surface each epibasidium usually narrows abruptly to form the relatively large sterigma 0.7 μ in diameter and 2 to 4 μ long (FIG. 3: 18, 19); less commonly the epibasidium protrudes 5 to 7 μ above the " jelly " surface before narrowing to the sterigma (FIG. 3: 32).

Nuclear migration in all three species usually begins at an early stage in the formation of the epibasidia. In its migration from the hypobasidium through the epibasidium, the nucleus undergoes very slight elongation until near the base of the sterigma (FIG. 1: 21, 23, 28; FIG. 2: 29, 31; FIG. 3: 22–24). While the nuclei of any basidium generally migrate simultaneously, at times, the greatest irregularity obtains, one nucleus being far behind the others (FIG. 3: 31) or, in other cases, one nucleus being in the spore before the others have left the hypobasidium (FIG. 2: 35). Great elongation of the nucleus necessarily occurs during its passage through the slender sterigma (FIG. 1: 41; FIG. 2: 36, 37). Once within the spore the nucleus immediately becomes spherical, and moves to its final position in the rapidly maturing spore (FIG. 1: 44; FIG. 2: 42, 45, 46; FIG. 3: 34, 37–40).

A tiny spherical enlargement at the apex of the sterigma is the first appearance of the spore: this tip, which appears as nuclear migration begins (FIG. 1: 27; FIG. 2: 31, 32; FIG. 3: 17), rapidly enlarges, remaining spherical and usually without dense protoplasmic contents, until the nucleus has migrated almost to the apex of the epibasidium (FIG. 1: 29, 30, 42; FIG. 2: 33; FIG. 3: 20, 21, 30). Then very definite elongation of the spore occurs, with continued increase in diameter, until the dimensions of the mature spore are attained (FIG. 1: 31–33, 41; FIG. 2: 36–39, 42, 45, 46; FIG. 3: 33, 37, 39).

Subsequent to the migration of the nucleus from the hypobasidium into and through the epibasidium, increasing vacuolation occurs, soon resulting in an absolutely empty basidium, as for example FIG. 1: 24, 25, 41; FIG. 2: 36–39, 45–46; FIG. 3: 22–24; in the very large basidia of *S. epigaea* this disappearance of protoplasm is especially noticeable, the withdrawal following close after the migration of the nucleus. In this species, also, complete collapse of the empty basidia soon occurs, rendering extremely difficult accurate tracing of the entire structure.

Soon after its maturity, the spore germinates by means of a single short lateral germ tube, which ends abruptly in a slender sterigma at the tip of which is formed a single secondary spore quite like but smaller than the primary spore (FIG. 1: 43, 45; FIG. 2: 43, 44). Into this secondary spore the single nucleus migrates.

In *S. deminuta,* one frequently finds, particularly near the outer margin of the fruit-body, irregular, spherical to fusiform terminal cells each containing a single nucleus about 1 μ in diameter (FIG. 1: 23). From the apical end of this cell a single tube from 2–7 μ long develops either upright to the substratum or extending out over its surface (FIG. 1: 46, 48). The end of this tube narrows to a slender sterigma at the end of which there forms a spore entirely like the normal basidiospore (FIG. 1: 47, 51).

In addition to the reproductive cells just described, there are in the fruit-bodies of *Sebacina* other objects which are distinct from the basidial initials in having a less dense protoplasmic content, and which also in many cases show a characteristic early branching. These structures, cystidia, gloeocystidia, paraphyses and dendrophyses, show at first little that is distinctive. They first appear as erect hyphal tips whose two nuclei occupy a position near the center, and finally side by side (FIG. 2: 34, 35, 38), if indeed they have not disintegrated prior to this. Further development shows definite differences distinguishing these objects. Growth of the gloeocystidium continues until it becomes an irregular tube 8–16 μ long and 2.0–2.5 μ in diameter, in which the protoplasm generally becomes progressively thinner while there accumulate increasing quantities of oil-like drops (FIG. 1: 37). This thinning of the protoplasm becomes more pronounced with further enlargement of the gloeocystidium, which now develops apically a long some-

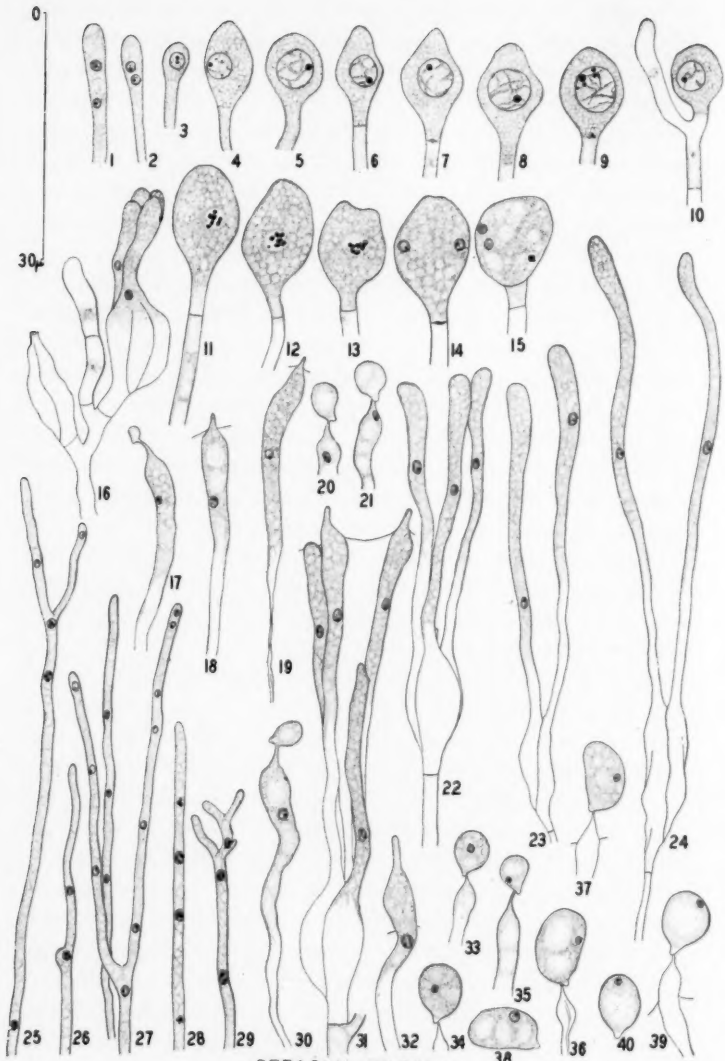

0
30
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
34
33
35
38
36
37
40
39
SEBACINA EPIGAEA

FIG. 3.

what crooked extension, at first seemingly devoid of protoplasm (FIG. 1: 34), but, when 6–7 μ long, is filled with a uniformly distributed but very thin protoplasmic content (FIG. 1: 52), and many drops of an oil-like substance. Nuclear activities during this apical elongation vary somewhat, but most commonly the two nuclei migrate into the apical portion where first one and then the other disintegrates, after which the protoplasm disappears, leaving a somewhat collapsed tube in which remain vestiges of protoplasm, generally as a faint ring of strand in the region of the junction of the apical portion with the basal (FIG. 1: 53).

Occasionally, even before this apical extension forms (FIG. 1: 37, 39), fusion of the two nuclei takes place, after which the various stages of division of the fusion nucleus (FIG. 1: 36) occur, even up to the formation of somewhat abnormal chromosomes (FIG. 1: 40). Sooner or later, however, the nucleus disintegrates. It is interesting to note that when fusion occurs there is a tendency towards swelling of the containing body, a swelling which is more pronounced the farther the nuclear development proceeds.

Early stages in the development of the cystidia in no way differ from those of gloeocystidia; indeed, were it not that individual species of *Sebacina* seem characterized by one or the other of the two objects, but not both, it would be impossible to distinguish them in early stages. Gloeocystidia long possess contents of "resinous" nature, whereas cystidia early become extremely vacuolate while at the same time they increase tremendously in size. As this development progresses, the two nuclei come to a position near the tapering tip of the cystidium (FIG. 2: 48), and there disintegrate successively, following which there is a disappearance of all protoplasm (FIG. 2: 47), until eventually a tapering somewhat bent body 25–55 μ long and up to 5 μ in diameter is developed. Frequently these objects extend upwards from the lowest stratum of the fruit-body to the "jelly" surface above which they may protrude as slender narrowing tips; in other fruit-bodies two or even three successive strata containing cystidia may be noted. All cystidia soon become devoid of protoplasm, and eventually collapse and are lost in the fruit-body. Certain lateral branches, which form paraphyses are associated with the hypobasidia of *S. epigaea*. These paraphyses become vacuolate even earlier in their develop-

ment than the two objects above described, the protoplasm remaining as a thin lining at the apex and an equally thin band in the middle of the segment. In this band may frequently be seen traces of the disintegrating nuclei (FIG. 3: 10). At times these objects become two or three septate (FIG. 3: 16), but always end as empty somewhat collapsed bodies.

There remains for final consideration those irregularly branched structures which have been called dendrophyses. These also have the same early development, but soon become irregularly much branched (FIG. 1: 49); and keep their dense protoplasmic content throughout their existence. Whether these are distinct objects characteristic of the fruit-bodies of *Sebacina* or merely an early stage in the branching hyphae of the enlarging fruit-body as is the case in fig. 3: 29, has not been determined. They often seem to be distinct objects.

DISCUSSION

Corresponding stages of development of the fruit-body in species of *Sebacina* show considerable similarity.

The hypobasidial initials, always binucleate in their earliest stages, form a definite hymenial layer over the exposed surface of the fruit-body. As this gets older, the hymenium becomes thicker and thicker, the basidia forming a dense mass in which no obvious separation of younger basidia as more superficial than the older can be made except in a most general way. In the development of each basidium there occurs an early fusion of the two primary basidial nuclei which seems to incite rapid and pronounced swelling of the hypobasidium. At the same time the fusion nucleus expands correspondingly, with characteristic changes. Most noticeable among these is the early organization of the chromatin into definite discrete strips located at the nuclear surface. These chromatin masses are longer and thinner in *Sebacina* than those noted in *Exidia* or *Tremella,* and seem to be six in number, instead of eight. During this nuclear enlargement, the nucleolus has remained a very conspicuous dark staining body located at the periphery of the nucleus. The mature fusion nucleus is essentially as described by Maire and Kühner, having very evident (Kühner) long slender chromatin threads (Maire) which are not

continuous. The present studies have not supported Neuhoff's observation that the chromatin is aggregated into two faintly staining nets.

The greatest differences exist in the descriptions of developments which occur during the formation of the chromosomes. Maire's observation (**2**) was that the chromatin becomes gathered into small masses, the protochromosomes, from which the two chromosomes are formed; Neuhoff (**3**) thought small definite chromosomes, four in number, were massed closely in the center of the hypobasidium; while Kühner (**1**) found two indistinct masses which he considered to be chromosomes. Present studies indicate that six very small but distinct chromosomes are formed; usually these are so compactly grouped as to render very difficult accurate counting of their number. An added difficulty is the frequent presence of one or two minute bodies with the chromosomes. Coincident with the formation of the chromosomes, the nucleolus and nuclear membrane have disappeared, while the transversely oriented spindle has appeared; this spindle was never a conspicuous object in any sections observed in the present study.

The two daughter nuclei at once take definite form and with no resting period may divide again, although quite commonly one or both of them fails to divide. The small size of these nuclei renders it difficult to follow accurately the changes during this division. It is evident, however, that there are three minute chromosomes which divide equationally, showing that the first division is the reduction division.

As Neuhoff (**3**) has pointed out the development of the epibasidia is remarkably uniform. The variations which do occur, such as the wide-spreading epibasidia of young fruit-bodies which contrast with the erect closely massed epibasidia formed later seem definitely to result from the increased "jelly" mass through which they grow. Emergence from this "jelly" mass seems to induce the development of sterigmata.

The formation of the septa has occurred at this time and the writer agrees with Neuhoff (**3**) that they develop very rapidly. Nor have the early stages of nuclear migration offered much of interest. Each nucleus elongates only slightly during the entire period of migration until its passage through the sterigma into

the spore, when, due to the minute diameter of the former, the nucleus necessarily becomes much elongated. However, immediately on entering the spore the spherical form is reassumed. Present studies support Neuhoff's statement (**3**) that the nucleus enters the spore only when the latter is nearly mature; this may be explained by the fact that nearly all the protoplasm has passed into the spore before the nucleus. There are cases, however, where the spore receives its nucleus before full-size is attained; subsequent increase in size is accompanied by increasing vacuolation of nuclear contents. Spore germination always gives rise to secondary spores entirely similar to the primary; no nuclear divisions have been noted in this germination.

Because of their debatable value as points for generic separations, the various non-basidial objects—cystidia, gloeocystidia, paraphyses, and dendrophyses—which are present in the hymenial layer were particularly noted in the present study. In their early stages these all seem to be identical, except that those objects interpreted as paraphyses seem mostly to be branched from the basidial "stalk." It is extremely difficult to decide when seen in its early development what a given hyphal tip eventually will become. Great increase in size and vacuolation of content accompanied by degeneration of the two nuclei determines the cystidia. Considerable accumulations of "oil"-like contents indicates a gloeocystidium. Present studies have not shown any prominent spiral bands within these such as Kühner (**1**) has figured and described. Such bands as occur are usually faint and irregular, and are probably vestiges of cytoplasm as Kühner considered them. Final development of gloeocystidia leads to the exhaustion of the "oil"-like content leaving an empty partially collapsed object resembling a small cystidium. Little need be said of paraphyses and dendrophyses, beyond pointing out that many times objects resembling such structures are obviously only stages in the development of basidia or are hyphal growth.

These sterile structures have been used to separate *Sebacina* into several genera, a point discussed by Rogers, who held them to be insufficient for generic separation, but satisfactory for subgeneric distinction. It is difficult to separate the minute *Tremel-*

lae, such as *T. Grilletii* Boud., from certain species of *Sebacina;* a resupinate fruit-body does not always obtain, young bodies being often discrete and *Tremella*-like. Texture is equally troublesome, for fruit-bodies of *Sebacina* vary from coriaceous through waxy to gelatinous, depending often on the degree of wetness. Nor do any differences in the structure of the fruit-body appear, unless it be in the sterile structures. So far as the writer's observations go these may help form a means of separation, but in themselves they are not sufficient. Certainly the septate, sterile objects described under *Tremella* (**5**) have not been observed in *Sebacina,* nor have those objects called cystidia or gloeocystidia been found in *Tremella.* Further critical study of additional species of both genera will be necessary before any definite statements may be made relative to their taxonomic value.

SUMMARY

The three species of *Sebacina* studied, *S. deminuta* Bourd., *S. fugacissima* Bourd. & Galz., and *S. epigaea* (Berk. & Br.) Bourd. & Galz., have shown the same uniformity of development as was noted in *Tremella* and *Exidia.* Those binucleate hyphal tips which are to become hypobasidia are to be distinguished at first by a somewhat denser protoplasmic content and by a slightly greater diameter. As development progresses the two nuclei fuse, subsequent to which a pronounced swelling of the hypobasidium occurs, accompanied by an equally pronounced enlargement of the fusion nucleus. From the very first the chromatin material of the latter is definitely aggregated in definite linear patches, apparently always six in number. When the maximum size of the fusion nucleus is reached, contraction of the chromatin masses commences. Coincident with this rapid contraction the nucleolus and also the nuclear membrane disappear. When contraction is complete there are six small chromosomes densely massed near the center of the hypobasidium. These chromosomes separate three and three in reduction division and migrate to the opposite poles of the inconspicuous, transversely oriented spindle. Subsequent to the organisation of the daughter nuclei a second division may occur, either of one or of both nuclei, so that a mature hypobasidium may have two, three, or four small nuclei.

The formation of the septa and the development of the epibasidia offer little that is noteworthy; the differences in size and direction of growth which have been noted are an aid to separation of species but are not in themselves sufficient therefor. The migration of the nucleus usually does not begin until the formation of the septa is complete. During migration only slight elongation of the nucleus occurs, until it reaches the base of the sterigma, at which time it becomes extremely elongate in order to pass through the narrow tube. Immediately after the passage of the nucleus, the basidium becomes devoid of protoplasm; in the very large basidia of *S. epigaea* this is particularly noticeable. The mature, uninucleate, vacuolate spores germinate by means of a single, lateral germ tube through which the nucleus passes without division into the secondary spore.

Because of their possible value as a means of generic separation the non-reproductive bodies—cystidia, gloeocystidia, paraphyses, and dendrophyses—were particularly noted. All seem to form from binucleate hyphal tips at first not materially unlike hypobasidia. Early in development, however, these sterile tips become more vacuolate and show their characteristic differences; cystidia increase tremendously in size, losing at the same time their nuclei and most of their protoplasm; gloeocystidia become filled with an "oil"-like substance, which, with increasing age, disappears leaving objects similar to but smaller than cystidia; paraphyses early become vacuolate and enucleate; dendrophyses branch irregularly. These bodies become lost in the further development of the fruitbody.

LITERATURE CITED

1. **Kühner, R.** Contribution à l'Etude des Hymenomycètes et specialement des Agaricacés. Le Botaniste **17**: 1–215. *pl. 1–4.* 1926.
2. **Maire, R.** Recherches cytologiques et taxonomiques sur les Basidiomycètes. Thèses pp. 1–209. *pl. 1–8.* 1902.
3. **Neuhoff, W.** Zytologie und systematische Stellung der Auriculariaceen und Tremellaceen. Bot. Arch. **8**: 250–297. *pl. 1–4.* 1924.
4. **Rogers, D. P.** Some noteworthy fungi from Iowa. Univ. of Iowa, Stud. Nat. Hist. **15**: 9–29. *pl. I–III.* 1933.
5. **Whelden, R. M.** Cytological studies of the Tremellaceae. Mycologia. In press. 1934.

EXPLANATION OF FIGURES

Fig. 1. *Sebacina deminuta* Bourd. This and all other figures have been drawn with the aid of a camera lucida, at a magnification of 3800 ×, and subsequently reduced to about 0.3, *i.e.* to a magnification of about 1150 ×. In addition, an absolute scale of dimension is included in all figures.

1, hyphal segment in fruit body; 2, young hypobasidium with the two minute nuclei before fusion; 3, 5, same with very small fusion nucleus; 4, same with fusion nucleus with two nucleoli still distinct; 6, same with both nuclei and nucleoli fused; 7, 8, hypobasidium showing enlarging fusion nucleus; 9, 10, mature hypobasidia with accompanying hyphal tip; 11, hypobasidium with prochromosomes in the nucleus; 12, same with six chromosomes in the nucleus; 13, same with six chromosomes free in the center; 14, same with transversely oriented spindle; 15, 16, same with daughter nuclei near the surface; 17, same with two daughter nuclei dividing simultaneously; 18, same with first longitudinal septum forming; 19, same with first longitudinal septum separating the two nuclei; 20, same with one nucleus dividing and two epibasidia well-developed; 21, 22, same with four nuclei but only two epibasidia; 23, group of hypobasidia showing normal hypobasidium with nuclear migration at left and abortive uninucleate body at right; 24, 25, vacuolate mature spores on empty collapsing basidia; 26, 28, 29, hypobasidia showing formation of the long epibasidia found in old fruit-bodies; 27, 30–32, epibasidia with stages in the formation of the spores; 33, spore receiving nucleus attenuated in passing through sterigma; 34, binucleate gloeocystidium with slender vacuolate tip; 35, 38, early stages in formation of gloeocystidia; 36, 37, 39, gloeocystidia showing the two nuclei fused; 40, swollen gloeocystidium with thin protoplasm and nucleus dividing; 41, mature basidium with nuclei about to enter the spores; 42, same with form characteristic of young fruit-bodies; 43, spore showing lateral germ tube at tip of which a secondary spore is forming; 44, mature spore, showing vacuolate content; 45, same with lateral germ tube ending in slender sterigma; 46–48, stages of development of uninucleate body shown in fig. 23; 49, tip of dendrophysis; 50, large erect binucleate sterile hair projecting above surface of old fruit-body; 51, spore forming from uninucleate body like that shown in fig. 23; 52, mature gloeocystidia surrounding mature hypobasidium; 53, three mature gloeocystidia showing traces of protoplasm in fine bands; 54, base of a gloeocystidium showing but one nucleus; 55, hypobasidium developing abnormally by two mycelial tubes, each of which receives one of the two non-fusing basidial nuclei.

Fig. 2. *Sebacina fugacissima* Bourd. & Galz. 1, 2, hypobasidial initials, before fusion of nuclei; 3–7, hypobasidia, fusion nucleus enlarging; 8–10, same, containing mature fusion nucleus; 11, hypobasidium, prochromosomes contracting; 12, 13, 16, hypobasidia, prochromosomes nearly fully contracted, nuclear membrane gone; 14, 15, 17, same, showing six chromosomes; 18–20, same, each containing two nuclei; 21, hypobasidium, with basal and first longitudinal septa complete; 22, same, with basal and first longitudinal septa forming; 23, same, with two daughter nuclei dividing simultaneously; 24, same, with one daughter nucleus divided, other dividing; 25, same, containing four daughter nuclei; 26, same, with four coarse epibasidia forming apically; 27, same, with four coarse epibasidia fully formed

and with a delayed nuclear division in the hypobasidium; 28–30, early stages in migration of nuclei from hypobasidia; 31–33, same, in formation of spores at tips of epibasidia; 34, 35, two basidia showing irregularities in time of formation of spores; 36, 37, basidia showing passage of nucleus through slender sterigma; 38, 39, epibasidia becoming vacuolate subsequent to migration of nuclei; 40, 41, branched hyphal tips occurring among basidia; 42, 45, 46, mature spores still on epibasidia; 43, mature spore germinating by short lateral tube; 44, secondary spore still attached to empty primary spore; 47, 49, mature cystidia.

Fig. 3. *Sebacina epigaea* (Berk. & Br.) Bourd. & Galz. 1, 2, hypobasidial initials, before fusion of the two nuclei; 3, hypobasidium containing fusion nucleus in which nucleoli are still distinct; 4–7, hypobasidia with enlarging fusion nuclei; 8, hypobasidium showing mature fusion nucleus; 9, same, in which contraction of prochromosomes has commenced; 10, same, from "stalk" of which a paraphysis has developed; 11, 12, 13, hypobasidia, each showing six small somewhat irregular chromosomes; 14, hypobasidium containing two daughter nuclei; 15, same, in which one daughter nucleus is dividing, while other has divided; 16, bit of hymenium, showing septate paraphysis and hypobasidium from apex of which four closely-massed coarse epibasidia are forming; 17–19, epibasidia, showing vacuolation and collapse after passage of nucleus; 20, 21, epibasidial tip, showing formation of spore at tip of short slender sterigma; 22–24, basidia showing empty condition after passage of nuclei along epibasidia; 25–28, hyphal tips of fruit-body in condition of active growth, showing many dividing nuclei, each having three chromosomes; 29, dendrophysis-like hyphal tip, in which dividing nuclei show three pairs of chromosomes splitting in anaphase; 30, spore forming at tip of unusually irregular epibasidium; 31, basidium, showing migration of one nucleus much delayed; 32, epibasidium ending in long cylindrical sterigma; 33–35, enlargement of spore after entrance of nucleus; 36, 37, 39, mature spores at tip of empty collapsing epibasidia; 38, 40, mature spores.

THE PERFECT STAGE OF PHOMOPSIS STEWARTII ON COSMOS[1]

ARTHUR L. HARRISON

(WITH 8 TEXT FIGURES)

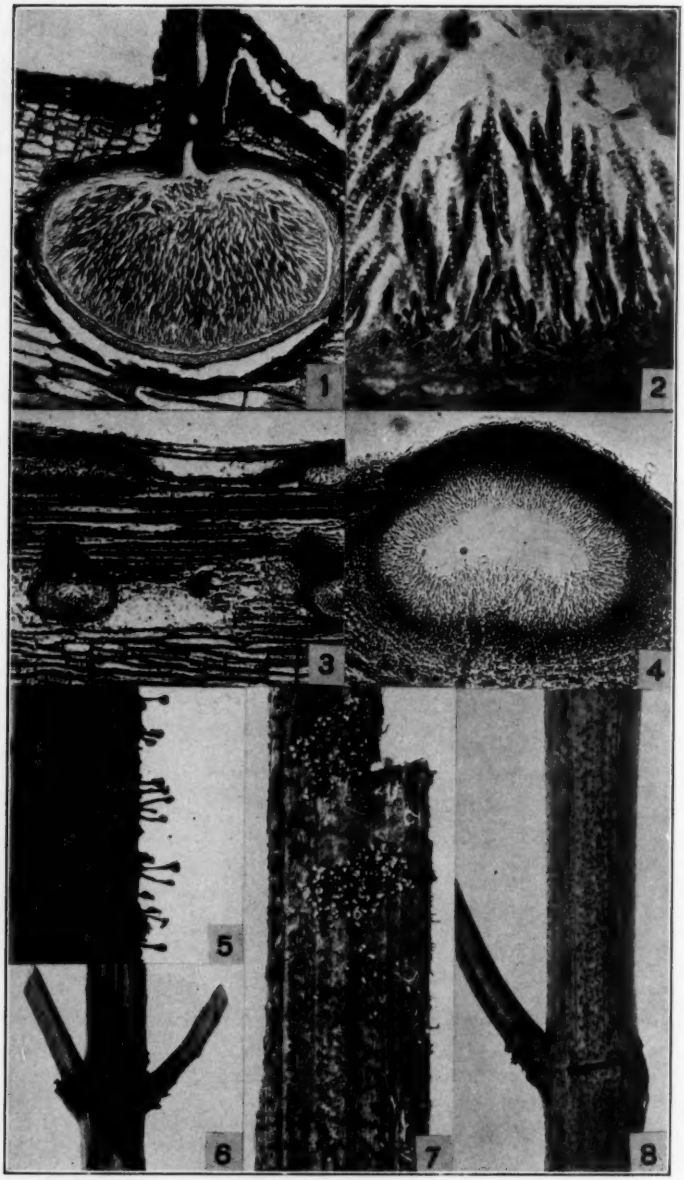
A disease of *Cosmos bipinnatus* L. to which the common name of stem blight is applied was recognized by Halsted (**2**) in New Jersey as early as 1894. Clinton (**1**) in 1903 reports its presence in Connecticut. Stewart (**6**) in 1910 states that the disease regularly makes its appearance in the *Cosmos* plantings at the New York State Experiment Station at Geneva. Professor H. H. Whetzel has informed the writer that it is present in his garden at Ithaca practically every year. In 1934 the writer observed that as many as 50 per cent of the plants were affected in the New York State Experiment Station plantings at Geneva.

Halsted (**2, 3, 4**) considered that stem blight of *Cosmos* is caused by a species of *Phlyctaena* since he apparently saw only filiform spores. Stewart (**6**), however, after observing these spores, which were stylospores, and pycnospores, sent the material to Peck (**5**) who described the fungus as *Phomopsis Stewartii* n. sp.

The material first used in the present study was supplied by Professor H. H. Whetzel from his garden in the autumn of 1932. In March of the following spring perithecia were observed on some of this material kept in a moist chamber for approximately five weeks. Since no perfect stage had been reported for *Phomopsis Stewartii*, the ascomycetous fungus was examined. It proved to be a species of *Diaporthe*. Since many species of *Diaporthe* have as their conidial stage a *Phomopsis*, the possibility that the *Diaporthe* and *Phomopsis Stewartii* were different stages of the

[1] Contribution from the Department of Plant Pathology, Cornell University. Part of the inoculation work was conducted at the New York State Agricultural Experiment Station at Geneva.

The writer is indebted to Professor H. H. Whetzel, Professor H. M. Fitzpatrick and Professor W. H. Burkholder for advice and criticism during the course of the investigation and for valuable corrections of the manuscript.



FIGS. 1–8. *Phomopsis Stewartii.*

same fungus was considered. Cultural work and inoculation experiments have demonstrated that such is the case.

The disease. Stem blight of cosmos is a disease of plants at approximately the blooming stage or of those weakened by parasites such as mildew or red spiders. The disease rarely makes its appearance until late August when the days shorten and the cosmos plants begin to bloom. The point of attack is usually at the nodes of the main stem or branches. Infection has not been observed on the leaves or roots.

The lesions first appear as dark brown areas, which rapidly enlarge until the stem is girdled. The parts above wilt and die. The lesions usually turn ashy gray and become dotted with numerous pycnidia visible to the eye.

ETIOLOGY

Isolations. Isolations of *Phomopsis Stewartii* were made by tissue and conidial plantings. Cultures of the *Diaporthe* were made from single asci obtained by placing small drops of an ascus suspension on the surface of agar plates. The drops were then examined under the microscope and those drops which contained a single ascus were marked. Later, after growth was visible, transfers were made to agar slants. Clonal lines of both the *Diaporthe* and *Phomposis Stewartii* were obtained by cutting off hyphal tips. Isolates from all sources appeared to be identical in culture.

Although Stewart states that *Phomopsis Stewartii* (**6**) does poorly on potato agar, growth is rapid on potato dextrose agar. *Cosmos* and *Melilotus* plugs and sterilized wheat kernels also have proved to be favorable media.

On potato dextrose agar the mycelium covered the petri dish in from 3 to 5 days when mycelium was planted, but in from 7 to 10 days when a single ascus or conidium was planted in the petri dish. Abundant whitish cottony aerial mycelium developed. Certain isolates produced a darkening of the agar.

Some isolates produced pycnidia abundantly on all media while others fruited very sparingly. Perithecia were produced in culture from only one series of isolations. These were on sterilized cosmos stems planted with single ascus cultures. Several other

single ascus cultures in the same series and in other series failed to develop perithecia. Likewise, all attempts failed to produce perithecia in culture from clonal isolates of ascospore and conidial mycelium, from non-clonal conidial mycelium, and from tissue plantings.

The fungus was reisolated from the inoculated plants. It appeared to be identical with the original culture used in making the inoculations.

Pathogenicity. The pathogenicity of the ascigerous and conidial stages was demonstrated in the greenhouse by inoculating cosmos plants with mycelium from tissue plantings, from both clonal and non-clonal lines of ascospore and of pycnospore cultures, and with ascospores and pycnospores from portions of cosmos stems in moist chambers. The mycelium was applied to the wounded surface of the cosmos stems at the nodes, and then covered with moist cotton and protected by lead foil about the stem. Checks with agar were used in most of the experiments. The ascospores and the pycnospores were applied in water suspension with an atomizer. The plants were then placed in a moist chamber for about 48 hours.

Infection resulted in 90 per cent (61 out of 68) of the cases on plants inoculated in bloom or in a weakened condition, while in only. 10 per cent (5 out of 46) on young plants. Each type of inoculum produced about the same percentage of infection. Infection did not occur in any of the 40 check plants. Old or weakened plants, in all cases, developed symptoms of stem blight much sooner than vigorously growing plants. The period of incubation and latent infection ranged from about two days to several weeks, depending on the age, size, and vigor of the cosmos plants.

Pycnidia were in evidence, in some cases, five days after inoculation. Perithecia have been observed repeatedly on portions of the artificially infected plants after these have been placed in moist chambers. In one case they developed within five weeks from the time of inoculation. Perithecia have never been observed in the field though no extensive search has been made for them. They have been produced once on diseased cosmos stems held under moist conditions under greenhouse benches. The *Diaporthe* under investigation is clearly, therefore, the perfect stage of *Phomopsis Stewartii* since cultures from *Phomopsis Stewartii* and the *Dia-*

porthe appear to be identical and since inoculations with both produce the disease.

Taxonomy. A careful study of the literature fails to reveal any mention of a species of *Diaporthe* on *Cosmos*. Material therefore was submitted to Dr. L. E. Wehmeyer. He states (letter of October 25, 1934) that "Morphologically, this material appears to be *Diaporthe Phaseolorum*, which is very close to *D. Arctii*, but has smaller spores, asci, and perithecia in general, and the ostioles tend to be finer and more elongate."

Under *D. Phaseolorum* Wehmeyer (**7**) includes several forms which have certain morphological and physiological characters in common. The *Diaporthe* on *Cosmos*, although similar in many respects to certain of the forms of *D. Phaseolorum*, differs in certain other respects. The long beaks, the narrow ascospores, and the small asci are characteristic of *D. Phaseolorum*, while the perithecia, pycnospores and length of the ascospores approach those of *D. Arctii*. Apparently, therefore, the *Diaporthe* in question occupies an intermediate position between *D. Phaseolorum* and *D. Arctii*. The writer feels that in consideration of the indefinite limits of the closely related species of *Diaporthe* a new species is involved. The name *Diaporthe Stewartii* is applied. The following description is based on the fungus as it occurs on *Cosmos*.

Diaporthe Stewartii sp. nov.

Synonym: *Phomopsis Stewartii* Peck.

Pycnidial stage. Pycnidia numerous, scattered, occasionally gregarious, simple or chambered, at first subepidermal, later erumpent, small, ⅓ to ½ mm. broad, black, provided with an ostiolar beak, 0–1 mm. long in culture or when affected *Cosmos* stalks are kept under moist conditions, beaks frequently hairy on surface near apex. Pycnospores common only in young pycnidia, oblong to subfusiform, hyaline, unicellular. 4.6–12.5 × 2.3–3.0 μ (average 6.6–9.2 × 2.3–3 μ). Sporophores slender equal to or shorter than the spore. Stylospores very common, filiform, curved, flexuous or uncinate, hyaline, 11.7–28.4 × 1.0–1.5 μ (average 16.7–21.7 × 1.0–1.5 μ). Both types of spore ooze through the ostioles in large quantities under moist conditions. For the original description see Peck (**5**).

Perithecial stage. On dead stems of *Cosmos*, single, scattered, embedded below pycnidial stromata, globose to lens-shaped, black,

308–600 × 252–420 μ. Stromata poorly developed. Beaks 1 to 1.5, rarely 2 mm. long, tapering to filiform, black with rounded tip, slightly hairy, apical pore circular. Asci sessile, elongate-clavate, apex thickened, with pore, oozing at maturity in masses through the long ostiole, 25.1–42.9 × 3.9–7.3 μ (average 31.7–37.6 × 4.6–5.9 μ), paraphyses evanescent. Ascospores biseriate, hyaline, ellipsoidal to fusoid, some slightly curved, bicellular, each cell biguttulate at maturity, 9.2–17.2 × 1.3–3.3 μ (average 10.6–13.2 × 2.0–2.5 μ).

Produces stem and branch canker on *Cosmos bipinnatus*. Pycnidia found on recently killed parts. Perithecia on same parts after 3–6 weeks in moist chamber. Type material in herbarium of the Department of Plant Pathology at Cornell University, Ithaca, New York, No. *21993*.

LITERATURE CITED

1. **Clinton, G. P.** Report of the Station Botanist. Conn. Agr. Exp. Sta. Rep. **1903**: 277–370. 1903.
2. **Halsted, B. D.** A serious blight of *Cosmos*. Garden and Forest **7**: 464–465. 1894.
3. ——. Report of the Botanist. New Jersey Agr. Exp. Sta. Rep. **1894**: 273–419. 1895.
4. ——. Report of the Botanist. New Jersey Agr. Exp. Sta. Rep. **1896**: 289–429. 1897.
5. **Peck, C. H.** Report of the State Botanist 1909. New York State Museum Bull. **139**: 1–114. 1910.
6. **Stewart, F. C.** Notes on New York plant diseases 1. New York (Geneva) Agr. Exp. Sta. Bull. **328**: 303–404. 1910.
7. **Wehmeyer, Lewis E.** The genus *Diaporthe* Nitschke and its segregates. Univ. Mich. Stud. Sci. Ser. **9**: I–X + 1–349. 1933.

EXPLANATION OF FIGURES

Fig. 1, a mature perithecium showing a portion of the filiform beak and numerous asci (× 80); 2, mature and young asci and ascospores, the mature asci with a distinct thickening at the apex (× 432); 3, young perithecia arising in tissue below the subepidermal pycnidia (× 80); 4, pycnidium lined with stylospores (× 168); 5, 7, masses of mature asci at the tips of the long perithecial beaks; 6, young lesion on *Cosmos* stem from artificial inoculation; 8, pycnidia on *Cosmos* stem from artificial inoculation.

TWIG BLIGHT OF THE AMERICAN BLADDER NUT CAUSED BY HYPOMYCES IPOMOEAE

W. H. Davis

(WITH 3 TEXT FIGURES)

A twig blight of bladder nut shrubs (*Staphylea trifolia* L.) was first noted during the summer of 1929 and seemed to be ubiquitous on shrubs planted for ornamental purposes in the Connecticut Valley. As the cause of this blight was unrecorded in the available literature, an investigation was undertaken to solve the following problems:

1. What is the cause of the blight?
2. During what season and where in the host is the disease initiated?
3. If a causal organism, how does it overwinter; has it other hosts?
4. What controls can be suggested for this disease, considering the data collected regarding the life history of the organism?

SYMPTOMS

The first symptom of this disease generally appeared during the last week in June, when the tips of young shoots showed infection in two or three adjacent internodes. At first, they appeared as if frozen since the dark ivy green bark changed to a velvet green, then a silvery gray and were somewhat curled. However, later in the season, the bark turned a purple drab color which was overlaid with the silvery waxy bloom of the stem. Afterwards, this bloom disappeared and the bark changed from an army brown to a silvery gray. The necrotic seasonal growth finally appeared blanched and a silvery gray. However, these color changes were not so pronounced in secondary infection of diseased stems which consisted of several seasons growth (FIG. 3*A*, *C*, *D*, *E*).

The bark shriveled in small, parallel furrows and remained attached during the greater part of the winter. In the spring, it often shredded and parted from the stem in small fibers. Then the white, solid, exposed wood could be observed. Furthermore, very little discoloration of the wood and pith was produced by the fungus.

The advance of the organism seemed to be arrested for a brief period when it reached a node, more especially during hot, dry weather. At the junction between infected and apparently healthy host tissue, a collar of purple brown or mouse gray, raised callous tissue was formed. Under the hand lens, this callous tissue seemed to be associated with the healthy bark and was separated from the necrotic bark by an apparent cracking of the dried necrotic tissue. When the fungus continued advancing down the twig, this collar remained behind so that a single twig sometimes bore several of these collars or terminal growth rings (FIG. 3*C*—1, *D*—3).

During a three-day period of warm, humid weather, spore masses or sporodochia appeared on the bark of diseased twigs (FIG. 3*C*—2, *G*). Sporodochia were a pinkish color when moist, but turned a creamy color when dried. They continued to form on recently infected areas during warm, humid weather in summer and in fall. Sporodochia also formed on diseased twigs which had overwintered and been exposed to warm, humid weather continuing for several days as on April 25, 1930, and May 18, 1932. However, conidia were more abundant on the sporodochia during the summer as on June 6, 1929, June 26, 1930, July 4, 1931, August 1, 1932 and July 15, 1933. Conidia not only formed on sporodochia, but on the surface of perithecia during the autumn and the spring as on September 14, 1932 and May 18, 1933.

The bark of old twigs sometimes changed from its brown color to an ashen gray and showed small, red, elevated flecks which indicated the position of the maturing perithecia. Later, the bark cracked and the dark, purple-red perithecia could be seen emerging through the parallel rifts. On old stems bearing secondary infection, the dead bark remained intact for several years, but only one generation of perithecia was formed in it (FIG. 3*E, F*).

The wood in old stems was not appreciably rotted by the or-

ganism, but appeared firm and highly resistant to stress and strain. However, the fungus lived in the bark and parenchyma of the main stem for several years before necrosis and perithecia appeared.

During a disease survey for the past two years, blighted shrubs were located in the Connecticut Valley extending from Vermont to Connecticut and west as far as Albany wherever bladder nut shrubs are cultured for ornamentation. Furthermore, two diseased shrubs were observed in an Ohio park and one in East Chicago, but none were located west of the Mississippi River.

PATHOLOGICAL ANATOMY

In buds, the hyphae penetrated between the scales and entered the meristem of the maturing twig. Stained, free-hand sections of this meristem showed intercellular and infracellular hyphae present. Furthermore, the fungus was cultured from the interior of buds collected during April 1932 which showed that the fungus overwintered in the buds (FIG. 1*A*, *B*, *G*, *H*).

In stems, germtubes not only entered the young lenticels, but sometimes penetrated the meristem of the twig tips directly. When the hyphae were within the stem, they readily penetrated pith cells and advanced rapidly through the medullary tissue along the main axis (FIG. 1). When meristem had formed wood tracheids, hyphae passed through the pits when advancing from pith to bark (FIG. 1*J*, *O*, *R*, *S*). In the bark, the parenchymatous tissue and the phloem were most often penetrated. However, hyphae were observed in the cambial layer and the medullary rays. When host tissue was collected in April 1932, disinfected and implanted on potato-dextrose agar, cultures of the fusarial type of the fungus were obtained from pith and bark. This showed that hyphae overwintered in infected twigs at the youngest collar and at the node above it. In the spring, hyphae often grew between the cells of the bark, reached the surface and sporulated during the first warm, wet weather (FIG. 1*E*, *B*). The leaves were not perceptibly parasitized and did not become infected when inoculated as did the stems.

THE FUNGUS AND ITS CULTURE

Several substrata were employed for culturing the fungus: potato-dextrose agar, corn meal mush, oatmeal, carrot decoction, raw and cooked plant parts consisting of rice, potato tuber, carrot root, bean pods; stems of melilot, asparagus and bladder nut. The

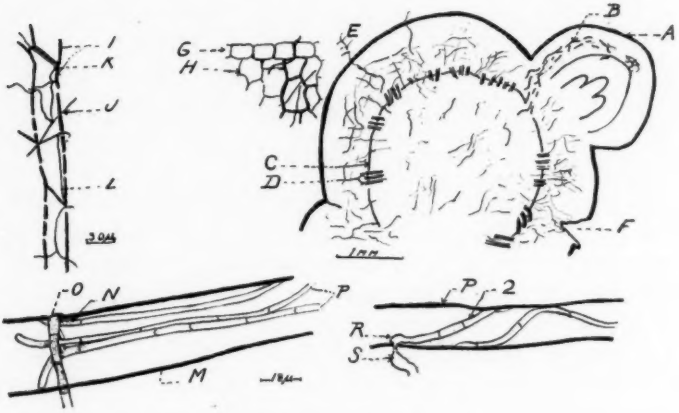


FIG. 1. *Hypomyces Ipomocae.*

fungus grew well and sporulated on potato-dextrose agar, but perithecia formed best on melilot, asparagus and bladder nut stems. Chlamydospores were observed in water cultures and in old agar cultures. Pure cultures were obtained by isolating individuals from macroconidia, perithecia, asci and ascospores. Here it should be stated that the microconidia failed to germinate in the cultures employed.

The acidity of the agar was determined by use of Fuller's scale because necessity demanded it. Twenty-five was the upper limit for mycelial growth; minus 32 for the lower or acid tolerance and minus 5 for maximum mycelial and conidial formation during short incubation periods. However, good mycelial growth and sporulation was observed in Petri dish cultures of agar adjusted to minus 25 F. S. and incubated at 22° C. for long periods.

The hyphae in stem tissues were 2 to 3.5 microns in diameter, averaging 3 microns; cross walls were present, but without con-

strictions, profusely branched in the phloem of the host, but without definite haustoria. In a three-day culture on potato-dextrose agar, pH 6.4 to 6.8, the mycelium was dark brown to black at the substratum, while at the surface, it was a burnt umber, van Dyke brown, or fawn color; yet, some areas showed a pinkish color (FIG. 3*J*, *K*). The growth was rather strict, seldom over 1 mm. above the surface of the agar; pinnotes and sporodochia formed on the host and in artificial cultures. These varied from a rose to a cream color, but when old and dried, they often turned a tint of brown. Yet, another monoascosporous culture showed a lemon hue which changed to rose-violet after incubating for 5 days. After 7 days, mycelium over the whole surface was purplish with a rose color at the center and a gray substratum which was somewhat pelicellate. These color changes were afterwards duplicated in another series. In general, variations in culture characters commonly associated and described for *Hypomyces* were observed in the cultures of this fungus.

Ascospores from perithecia collected in February were set to germinate in van Tieghem cells using potato-dextrose agar as a substratum. The perithecia were first washed in sterile water, then submerged in an aqueous solution of bichloride of mercury, 1:1000 for 30 seconds, washed in two changes of sterile water, a spore dilution prepared, and hanging drops placed on agar-smeared cover glasses so as to form van Tieghem cells which were then stored in a damp chamber for incubation at 22° C. After incubating for seven days, microconidia were formed and in three weeks both microconidia and macroconidia were present in a ratio of 10 to 1 (FIG. 2*A*, *B*, *C*, *D*, *E*, *F*).

Microconidia were hyaline, oblong-cylindrical, and abstricted from the ends of side branches (conidiophores) on the germtubes of germinating ascospores and other hyphae. They formed best in carrot decoction cultures of germinated ascospores which had been incubated for 24 to 48 hours at 22° C. (FIG. 2*A*. June 10, 1931.). Microconidia were repeatedly abstricted from the tips of conidiophores and sometimes remained in contact with each other, arranged side by side while at other times, in a somewhat globose mass or " false head " surrounded by water (FIG. 2*B* and *C*).

The sizes and shapes of microconidia varied according to the substrata employed. Two sizes were observed; the large, globose, cylindrical type and the small, often alantoid-cylindrical type. Each of these was produced from one monoascous culture which

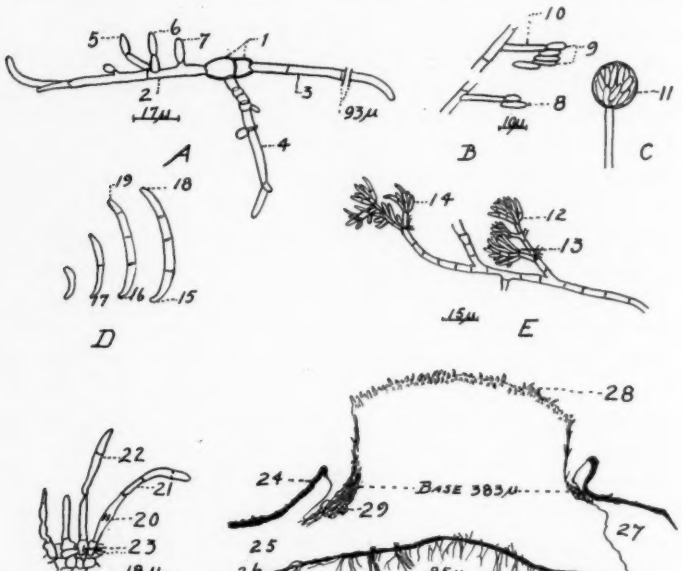


FIG. 2. *Hypomyces Ipomoeae.*

was transferred to different culture media and incubated three days. However, microconidia were cylindrical in most of the cultures.

The standard size for microconidia from the bladder nut as a host was 2.4 × 6.8 microns; 50 measured.

From the above data it is to be noted that the longest microconidia were cultured in carrot decoction and from germinated ascospores; they were smallest when cultured from the stroma as in No. 4. Only one lot, No. 7, showed an average width of 3 or more microns and only one lot, No. 3, showed a length over 7

microns. The average size in culture media was 2.3 × 6.8 microns as compared with 2.8 × 6.8 microns when removed from the host. The limits of variation for all measurements made were 2–4 × 5–10 microns (604 measured) which, considering the various substrata, compares favorably with those given by Wollenweber (**8**), 3–4.75 × 6–12 microns for *Hypomyces Ipomoeae*.

TABLE 1

MEASUREMENTS OF MICROCONIDIA COLLECTED FROM DIFFERENT SOURCES AND CULTURED ON POTATO-DEXTROSE AGAR (*PDA*) AND ON CARROT DECOCTION (*CD*)

Culture number	Culture medium	Source of inoculum	Measurements (microns)
1	*PDA*	Monosporous isolations	2 × 6.8
2	*CD*	2d transfer; monosp. culture	2.5 × 6.
3	*CD*	Ascospores; germtubes	2 × 10
4	*CD*	Pycnidiosclerotium—stroma	2 × 6
5	*PDA*	Host bark	2 × 7
6	*PDA*	Culture	2.5 × 6
7	*PDA*	Perithecium attached to host	3.4 × 6.8

Macroconidia were obtained in monoascosporous cultures following the production of microconidia. They were especially prevalent on hyphae in cultures isolated from germinating ascospores and often found in sporodochia which formed on the surface of infected twigs. They also formed on perithecia, on infected young wood, on perithecioscleroti a (stroma) located at the base of empty perithecia, and in pinnotes on agar cultures. In cultures, they formed after an incubation period varying from a minimum of two weeks to a maximum of five weeks. However, a monomacroconidial culture isolated on April 16 and incubated for 4.5 weeks contained sporodochia with both micro- and macroconidia. The macroconidia were of the fusarial type, mostly triseptate to quinseptate. However, spores with 0, 1, 2, 3, 4, 5, 6, and 7 septa were observed. The shapes were similar to those described by Wollenweber (**8**); nearly cylindrical, septum zone slightly pointed, and curved ends. Base pedicellate without a distinct keel (FIG. 2*D*, *E*, *F*, *G*). The measurements in microns of fifty macroconidia in each group follow:

Group	Writer	Wollenweber, for H. Ipomoeae
3-septate	3.8 × 31	3.75–5 × 30–45
5-septate	4.0 × 56	4.25–5.5 × 45–70
7-septate	5 × 68	6 × 70

It is to be noted that the measurements of these macroconidia observed in culture compare favorably with those described by Wollenweber for *H. Ipomoeae* (**8**).

Chlamydospores were observed in agar cultures which had been prepared in November 1931 and incubated until the following March. They were scattered among plectenchymatic pellicles. This experiment was duplicated during the next year with identical results. It was also noted that when transfers were made from the chlamydosporous cultures, cultures of like type were obtained and not typical microsporous and macrosporous cultures as might be expected. Chlamydospores also formed sparingly in water cultures after incubating for one or more months. They were globose to slightly elliptical, mostly terminal and unicellular, averaging 9 microns in diameter. Wollenweber (**8**) describes similar chlamydospores for *H. Ipomoeae*.

Perithecia were observed on a major percentage of old infected bladder nut shrubs (FIG. 3*H*). From 1931 to 1933 inclusive, they made their first appearance during September, but expelled their ascospores during the following spring and summer. Perithecia were mostly gregarious; the groups averaged 0.8 mm. in length; ten of the largest groups averaged 35 perithecia each, but one group contained the maximum of 75 perithecia. Furthermore, individual perithecia were observed on bark and wood which had been exposed by wounding. The majority of the perithecia emerged through slits in the bark surrounding necrotic stems which were several years of age. Perithecia were formed on a plectenchymatic stroma in which their bases were slightly sunken (FIG. 3*H*). When first formed, they were a reddish-brown, but finally became much darker, often appearing a brown black. They were ovoid and their sizes were exceptionally variable as can be seen from the following table.

It is to be noted that the sizes of perithecia fluctuate and that this fluctuation is associated with the seasonal development. Wollenweber stated (**8**) that there is a considerable fluctuation in the average sizes of perithecia. However, measurements slightly smaller than this standard are not considered sufficient to eliminate the perithecia measured from the genus *Hypomyces*.

TABLE 2

LIMITS OF VARIATION FOR THE MEASUREMENTS OF 50 PERITHECIA COLLECTED FROM MATERIALS UNDER DIFFERENT ENVIRONMENTS

No.	Conditions	Limits of variation (microns)	Measurements of Wollenweber
1	Spring—outdoors	126–361 × 168–420	
2	Winter—outdoors	151–235 × 150–259	
3	Herbarium—dried	128–238 × 150–272	
4	Standard—all	155–278 × 156–317	175–300 × 225–375
5	Absolute fluctuation	126–361 × 150–420	150–350 × 200–425

The exoperithecium was verrucose, but not so abruptly as generally illustrated for *Hypomyces Ipomoeae* (FIG. 3*H*), but the surface of these cells often possessed the characteristic glassy luster or sheen. The endoperithecial wall consisted of minute, hyaline hyphae extending somewhat parallel to the exterior surface and some of these hyphae emerged through the ostiolum functioning as periphyses (FIG. 3*H*). Scattered paraphyses were formed among the asci at the base of the perithecium (FIG. 3*I*). Some big club-shaped cells, with large granules within, were scattered among asci and considered as abortive asci.

Asci were hyaline with a blue tinge, clavate, somewhat blunt at the apices and narrowed toward a basal cell from which they seemed to project. Limits of variation in sizes of asci follow: 5–12 × 72–94 microns; standard, 9 × 82 microns (100 measured).

Each matured, normal ascus contained eight ascospores generally arranged uniseriately (FIG. 3*I*). In general, these ascospores compared favorably with those described by Wollenweber for *Hypomyces Ipomoeae* except that *the exosporia were not striated*. Strict search of fresh and of herbarial materials was made, the specimens mounted in water and in lacto-phenol-green and oil immersion objectives with Zeiss lenses employed, but *no striations could be detected*. The measurements of ripe ascospores fluctuated only slightly (**8**) from those described by Wollenweber for *Hypomyces Ipomoeae* (**8**), 4.6–6.1 × 10–13 microns as compared with his standard of 4.5–6 × 10–13. Thus the sizes of ascospores compared favorably with Wollenweber's, but no striations were detected.

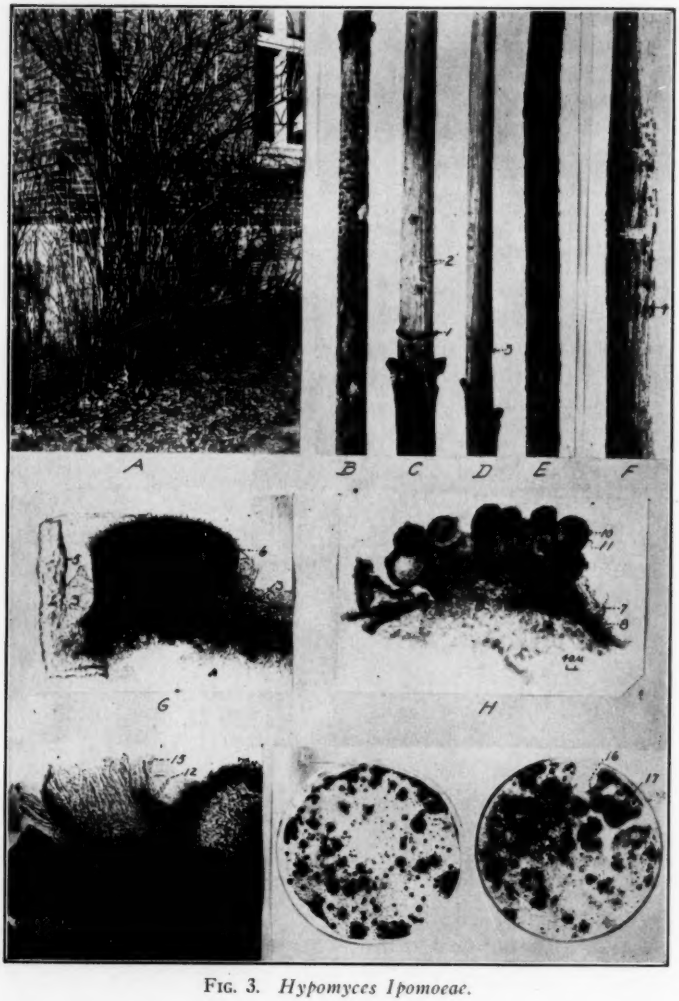


FIG. 3. *Hypomyces Ipomoeae.*

SYNONYMY AND CLASSIFICATION

Wollenweber (**8**) has presented the synonyms under which *Hypomyces Ipomoeae* has been reported. Berkley, Halsted and others reported it as a *Nectria,* but Wollenweber (**7**) changed it

to the genus *Hypomyces* since the ascospores are uniseptate and the polymorphic fungus bears other structures, previously mentioned, which are typical of the genus *Hypomyces*. However, contrary to Halsted's (**3**) findings and in accordance with Harter (**4**) this fungus (from the bladder nut) did not infect the sweet potato tuber. Furthermore, inoculations in fruits and stems of the eggplant failed to show infection.

Morphologically, the perithecia were of a darker color, their exterior walls less rugose, smoother, and the ascospores not striated. Otherwise, the parasite on *Staphylea* and *H. Ipomoeae* seemed sufficiently identical to be considered under one Latin binomial, but of a different forma so *Hypomyces Ipomoeae* (Hals.) Wollenw. forma *Staphyleae* is suggested for the parasite.[1]

INOCULATIONS

Living parts of the bladder nut were inoculated in the field together with excised living parts which were stored in the laboratory. The inoculum consisted of a sterile water suspension containing microconidia, macroconidia and mycelium from test tube cultures; also, germinated and ungerminated ascospores of *Hypomyces Ipomoeae* isolated from the bladder nut. The usual precautions for disinfecting surfaces—covering with glassine bags and with cellophane cylinders containing damp cotton or sphagnum moss—together with the proper preparation of checks were observed.

The laboratory inoculations were made during May, June, July and September so as to employ twigs, buds and leaves in different stages of development and under different environmental conditions. Excised twigs bearing leaves were disinfected by submerging them in an aqueous solution of mercuric bichloride for two minutes, washed in sterile water, then placed in 18-inch sterile glass jars so that the lower cut ends were buried in wet cotton. After the inoculations were made, the jars were sealed and stored at room temperature for six to twelve months. The air was suf-

[1] Monoascosporous and monomacroconidial cultures containing macroconidia were sent to Doctors Sherbakoff and Wollenweber. Each reported the fungus as *Hypomyces Ipomoeae*. The writer appreciates their aid in this classification.

ficiently changed during examination in an inoculating cage. This experiment was repeated in 1932 and 1933 with a total of eight separate experiments. The results showed:

1. The inoculated leaves did not become infected.
2. The wood was not perceptibly rotted.
3. The fungus produced a bark disease thereby girdling the young twigs and partially destroying the bark.
4. Microconidia, macroconidia, and perithecia formed on the diseased bark.
5. Old wood and old bark were not infected.
6. Germtubes from ascospores and macroconidia with mycelium infected young twigs so as to artificially produce symptoms identical with natural infections observed in the field.
7. Most perithecia had carbonaceous walls, were filled with oil globules and paraphysis-like hyphae, but one inoculation on May 18, 1932, produced ascospores after the culture had been incubated for six months.

Field inoculations were made during the months of April, May, June, July and September in 1931 and 1932. Bark and wood of old stems and young twigs, opening buds, closed buds, leaves, flowers and fruits were inoculated.

The best infection was obtained by an inoculation made on May 18, 1932. The inoculated young twigs showed infection in July and sporulation was noted on the surface of the bark. However, one twig did not show the symptoms until 1933, or about 14 months after inoculation. Both September inoculations failed to show infection but on June 18, 1931, an inoculation was made by placing macroconidia on opening buds, and 80 per cent infection resulted while no infection was obtained in old stems punctured and in wood inoculated. Punctured tips of young twigs showed 60 per cent infection while the unpunctured showed but 2 per cent infection. From inoculations made in the open, identical results were obtained as from those made indoors. However, infection may not become evident until the next summer following the inoculation. Furthermore, infection may cause cankers on the twigs and puncturing considerably aids infection. Opening buds and the meristem at the tip of young twigs afforded the best tissues for

entrance of the germtube. Old wood and bark were not infected. The wounds remained open, but in a good healing condition.

Data regarding the seasonal development of the disease were obtained from the inoculations and from observations in the field. In the spring time, hyphae from conidia and from ascospores penetrated the meristematic tissues in buds and in the tips of immature twigs. Infection was aided by the punctures of insects and by other wounds, but hindered by hot dry weather if not entirely prevented. A wet, warm environment was necessary for the entrance of the germtube into the meristematic tissues. About sixty days after inoculation, new conidia may appear on the infected stems and serve as a new source of inoculum.

The seasonal development as observed in the field and laboratory may be briefly stated. In the springtime, the germtubes penetrate the meristem of stems and opening buds; then, their hyphae advance downward through the young nodes and internodes until their activities are arrested by unfavorable conditions brought about by the maturing of the wood or the dry weather. Here a collar is formed in an internode and the mycelium may overwinter in the phloem and form conidia during the following spring or continue its growth during favorable conditions in late summer or autumn. Infected twig tips become necrotic and the organism advances from these tips to the older stems which may not be killed for several seasons. During the first or the second year of infection, perithecia form on the old necrotic twigs during the autumn, but only one generation of perithecia form in a given bark area.

The ascospores emerged in the spring, but did not remain viable longer than 11 months in herbarium materials and did not germinate later than August when collected from hosts in the open. Macrospores, ascospores and hyphae overwintered while attached to the host in the open and were viable in the spring of 1932. The disease was confined to the bark and the buds since leaves, floral parts, wood and fruits were not infected when inoculated. The most severe damage occurred in the young root-sprouts which sooner or later should form new stems and plants. The new stems emerged from the soil, remained in the shade under the canopy of the shrub, where conditions were generally ideal for infection

from the conidia and ascospores which fell from the infected areas above them.

CONTROLS

From a study of the disease and life history of the parasite, the following controls are suggested:

1. In June and July, the diseased tips of young twigs should be pruned below infected nodes and the infected materials burned at once since they bear numerous macroconidia which are potentially capable of disseminating the organism and disease.

2. In November, when the leaves have been shed, all old diseased canes should be removed and burned since perithecia form in the bark of old, diseased canes and overwinter there.

3. Prune so as to open up the shrub branches and allow air to circulate more freely around the branches and delay or often prevent infection.

4. Aphids, beetles and other insects often parasitize this shrub and disseminate the fungus. For this reason, parasitized bladder nut shrubs should be sprayed with arsenicals and with other insecticides so as to keep them free from insect vectors.

5. It seems reasonable to suppose that copper dusts and sprays applied from May to August would control the parasite since infection and spore emission take place during these months.

SUMMARY

1. *Hypomyces Ipomoeae* caused a twig blight of the American bladder nut.

2. The disease is initiated during the spring and summer when germinating ascospores and macroconidia penetrated meristematic tissues. Secondary infection occurs by the hyphae advancing through the phloem into the older twigs.

3. The organism overwinters as ascospores, macroconidia and hyphae within the bladder nut. No other host was observed.

4. Five controls are suggested in keeping with the life history of the fungus and seasonal development of the disease.

DEPT. OF BOTANY,
MASS. STATE COLLEGE,
AMHERST, MASS.

LITERATURE CITED

1. **Appel, O. & Wollenweber, H. W.** Grundlagen einer Monographie der Gattung *Fusarium* (Link). Arb. Kaiserl. Biol. Anstalt. **8**: 1–207. 1913.
2. **Brown, W.** Studies in the genus *Fusarium* VI. Ann. Bot. **42**: 285–304. 1928.
3. **Halsted, B. D.** Report of the botanical department; the eggplant stem rot. Ann. Rep. N. J. Agr. Exp. Sta. **12**: 281. 1891.
4. **Harter, L. L. & Weimer, J. L.** A monographic study of sweet potato diseases and their control. Tech. Bull. U. S. Dept. Agr. **99**: 77. 1929.
5. **Plowright, C. B.** A monograph of the British *Hypomyces*. Grevillea **11**: 1–8; 41–51. 1882.
6. **Sherbakoff, C. D.** Fusaria of potatoes. Mem. N. Y. Agr. Exp. Sta. (Cornell) **6**: 87–270. 1915.
7. **Wollenweber, H. W.** Studies on the *Fusarium* problem. Phytopathology **3**: 24–50. 1913.
8. ——. Identification of species of *Fusarium* occurring on the sweet potato, *Ipomoea Batatas*. Jour. Agr. Res. **2**: 270–281. 1914.
9. ——, **Sherbakoff, C. D., Reinking, O. A., Johann, Helen, & Bailey, Alice.** Fundamentals for taxonomic studies of *Fusarium*. Jour. Agr. Res. **30**: 843. 1925.

EXPLANATION OF FIGURES

Fig. 1. Tracings from camera lucida images showing the location of mycelium in stem tissues. The free-hand sections of infected bladder nut stems collected in the open were made and stained with lacto-phenol-green, washed with and mounted in clear lacto-phenol. (The drawings are partially diagrammatic.) *A*, a young infected bud in July condition; *B*, hyphae in the primordial scale tissue; also, in the bark where it tends to form radiations from the cambium; also, from the center in the pith cells; *C*, cambium; *D*, tracheids; *E*, mycelium emerging to the surface; *F*, lenticel showing hyphae of a primary infection in the bark beneath; *G*, hyphae within bud scale tissues; *H*, meristem penetrated by hyphae; *I*, tracheids bearing hyphae, *K* and *L*; *J*, hypha threaded through a pit in the tracheal wall; *M*, an enlarged tracheid showing a hypha passing through a pit at *O*; *N*, infracellular hypha; *P*, a tracheid showing that the hypha *Q*, has formed a penetrating tube between *R* and *S*.

Fig. 2. Drawings from camera lucida images showing the formation of microconidia and macroconidia in monosporous cultures.

A. An ascospore incubated for 24 hours (6–10–31) on potato-dextrose agar. Tracings of a camera lucida image. 1, two cells of the germinating ascospore; 2–4, germtubes; 5–7, microconidia.

B. Microconidia from a culture incubated for 72 hours. 8, microconidia excised from the conidiophore tend to stick together in a group; 9, a group of microconidia from one conidiophore; 10, conidiophore.

C. A false conidial head. 11, group of microconidia in a drop of water.

D. Macroconidia of various sizes and different numbers of crosswalls removed from a culture incubated for 40 days on potato-dextrose agar. 15–17, tips showing a greater curve; 18–19, hilum of spores with a greater curve at the base; the 4-celled, 3.4 × 30.6 and 3.4 × 29; 2-celled, 3.2 × 17; unicellular, 2.4 × 7.

E. Macroconidia forming on a hypha in culture; incubated for 3 weeks then dried (some conidia drawn free-hand). 12–14, macroconidia on the hyphae.

F. Macroconidia formed on conidiophores in a sporodochium from the host. Hand sectioned, stained with lacto-phenol, outlined image from a camera lucida. 20, conidiophore; 21–22, macroconidia in place; 23, foot cells and stromatic cells.

G. Diagram of a vertical, hand section through a sporodochium in the bark of the host (camera lucida employed). 24, dead bark of the host; 25, layer of infected bark; 26, cambial layer with hyphae projecting below; 27, epidermal layer, surface view; 28, tips of macroconidia crowded in the sporodochium, height, 213 microns; 29, a stroma of small, rhomboid cells formed below the basal line.

Fig. 3. Photographs showing symptoms of the twig blight and structure of the fungus, Hypomyces Ipomoeae, which infects the American bladder nut. *A*, a shrub showing numerous white tips of infected twigs. The large, diseased central leader which had been killed, was removed (3-2-'32); *B*, a twig infected with corynose twig blight. The unbleached condition and large, dark sclerotial areas bearing acervuli of *Coryneum* are to be contrasted with the perithecia of *Hypomyces* as shown in *E* and *F* (11-26-'31); *C* and *D*, white tips of infected twigs, at distal nodes, bearing sporodochia at *C*-2, and collars at 1 and 3; *E*, perithecia emerging through the bark of an artificially inoculated twig (11-26-'31); F, perithecia, 4, on a two-year necrotic stem; *G*, a sporodochium (12-12-'33), the epidermis of the host, 3; tisssue with macroconidia projecting above, 6; diameter 333 microns; *H*, section of a stroma and nine perithecia in host tissue. Outer layers of bark, 7–8; stromatic tissue, 9; outer wall of a perithecium, 10; inner wall, 11; asci, at the center; *I*, section through a perithecium highly magnified. 12, young ascus with uniseriated ascospores; 13, ascus wall and uniseptated ascospores; 14, paraphyses; *J*, petri dish containing a culture incubated 4 weeks. The dark areas show the locations of stromatic tissue; *K*, similar to *J*, only incubated two months and perithecia formed at 16, 17.

DICHOTOMOPHTHORA PORTULACAE, A PATHOGENE OF PORTULACA OLERACEA [1]

F. P. Mehrlich and H. M. Fitzpatrick

(with 3 text figures)

The well known weed, *Portulaca oleracea* L., is perennial in Hawaii and is abundantly distributed throughout the drier pineapple growing areas on all of the islands. Here it attains a size not known on the mainland of the United States. Single plants may cover a square meter of soil and individual stems may attain a diameter exceeding 2 centimeters.

Because of the facility with which it reestablishes itself from pieces of stem and owing to the vast number of seeds produced, it is the most difficult weed to eradicate from the fields, with the exception of nut grass, *Cyperus rotundus* L.

During the course of investigations to determine the possibility of controlling the weed by artificially disseminating naturally occurring pathogenes, it was learned that epiphytotics were being caused by an apparently undescribed hyphomycetous fungus.

The present paper is a description of this new organism. Another paper to be published elsewhere will discuss the epiphytology of the disease, and efforts to control purslane by its use.

DISTRIBUTION

The organism was first isolated during 1932 from diseased purslane growing in the dry regions of the island of Maui, extending ten miles from Makawao to Pulehu. No other areas of this island were sampled.

On the island of Oahu the pathogene was first found occurring over several hundred acres in the extensive dry areas of Robinson and Kunia. It was later found at Wahiawa, Kupehau, Sanitary

[1] Published with the approval of the Director as Technical Paper No. 83 of the Pineapple Experiment Station of the University of Hawaii.

Flats, Kahuku, Mokapuu, Koko Head, and Honolulu. None was found at Kemoo.

It has also been found on the islands of Lanai and Molokai, but not on the island of Kauai.

MORPHOLOGY

The pathogene is distributed intracellularly throughout all tissues of the stem and leaves of the host, forming ramifying brown hyphae approximately 6 μ in diameter.

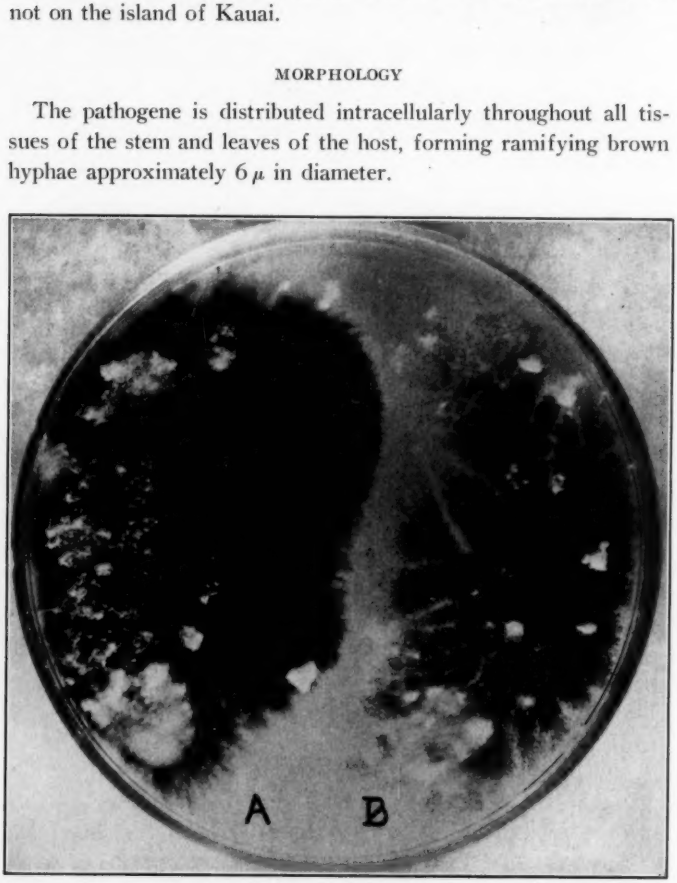


FIG. 1. A 14 day colony of A, strain L–34 and B, strain E–33 of *Dichotomophthora Portulacae* grown on malt agar.

On Difco corn meal agar, pH 6.4, the copious mycelium is at first hyaline and largely aseptate, in habit of branching resembling *Rhizoctonia* on the same medium. With age it becomes olivaceous

to black, divided into short cells, the hyphae anastomosing freely. The principal hyphae have a diameter of 6.5 μ, while the side branches are 5.0 μ or smaller. Neither pycnidia nor stromata are formed.

At least two distinct strains have been recognized which differ in size of conidiophores and conidia, also in character of growth on artificial media. In text figure 1 a photograph is given of a 14 day culture (A) of strain L–34 and (B) of strain E–33 on malt agar.[2] The rates of growth of the two are nearly the same. Strain L–34 produces conidia profusely but no sclerotia on this agar, while E–33 produces sclerotia[3] abundantly but conidia following desiccation only.

On *Portulaca* both produce abundant conidia, differing as described below, upon conidiophores which differ only in size.

The conidiophores are olivaceous to brown with the branching regularly dichotomous to subdichotomous in type (FIG. 2). The terminal cells of the ultimate branches are 4–8-lobed bearing a single conidium terminally on each lobe. As many as 60–75 conidia may be borne on a single conidiophore, a single branch producing as many as 14. The branches of the conidiophore occur in more than one plane in such a manner as to suggest a cyme.

As shown in text figure 2*c*, the conidiophores may repeatedly elongate. The lobed terminal cell while maturing spores may produce a lateral lobe which elongates, in turn producing a terminal cell, a new cluster of conidia and additional elongation, as described.

The conidiophores of strain E–33 measure 75–100 $\times$ 8–10 μ on malt agar. On plant material they range from 50–220 $\times$ 5–6.5 μ; average approximately 125 $\times$ 5 μ. The conidiophores of strain L–34 are significantly longer, ranging from 125–285 $\times$ 4–5 μ; average approximately 220 $\times$ 5 μ.

The conidia are dark brown, rarely slightly curved, ovoid to elongate-ovoid; in appearance superficially resembling the spores of Helminthosporium. They are one- to six-celled.

[2] Mehrlich, F. P. Medium for growth of pythiaceous fungi. Phytopathology **24**: 1127–1128. 1934.

[3] Such structures are termed bulbils by Whetzel in that they germinate by germ tubes only, true sclerotia giving rise to fruiting structures.

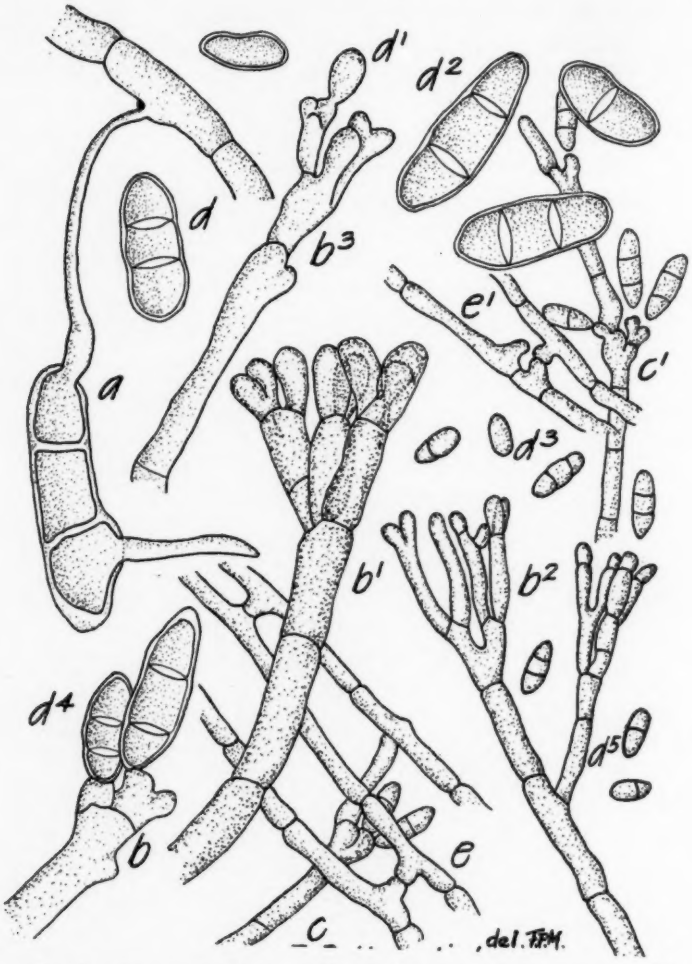


FIG. 2. The conidiophores, conidia, and anastomosing hyphae of *Dichotomophthora Portulacae: a*, germinating conidium showing a germ tube fusing with the parent mycelium, × 1200; *b*, b^1, b^2 (× 500), b^3, portions of conidiophores showing characteristic branching and mode of conidium attachment, × 1200; *c*, c^1, portions of conidiophores showing successive elongation, × 500; *d*, d^1, d^2, d^3 (× 500), d^4, d^5 (× 500), various conidia, illustrating 1 to 3 celled types; *e*, e^1, anastomosing hyphae, × 500.

TABLE 1

THE PERCENTAGE DISTRIBUTION OF CONIDIAL TYPES OF STRAINS E–33 AND L–34 OCCURRING ON MALT AGAR AND *Portulaca oleracea*

Spore classes	Strain E–33		Strain L–34	
	Malt agar	*P. oleracea*	Malt agar	*P. oleracea*
1-celled	2.3	0.0	0.0	7.4
2-celled	5.8	2.2	24.1	37.4
3-celled	46.5	15.7	72.8	51.6
4-celled	43.0	70.4	3.1	3.7
5-celled	2.3	10.0	0.0	0.0
6-celled	0.0	1.7	0.0	0.0
Total spores in sample	86	230	195	190

In table 1 is presented the incidence of the several spore types in randomly selected samples from malt agar colonies and from plant lesions resulting from pure cultures of the two strains. Note that the greatest number of spores of strain E–33 are of the 3- and 4-celled types, whereas the majority of those of strain L–34 are of the 2- and 3-celled types. In strain L–34 there are almost no 4-celled spores and no 5- or 6-celled types. Similarly strain E–33 produces almost no 2-celled forms.

These differences are considered to be significant, since the values given are for pure cultures of the two strains grown simultaneously under similar conditions.

As shown by table 2, the size of the conidia of strain E–33 is much larger than of L–34.

Despite these differences, the essential morphology of the two strains is so similar that they are regarded as a single species.

The conidia germinate by one or more terminal or lateral germ tubes. In culture the germ tubes of adjacent spores often anastomose with each other, or fuse with the parent mycelium. In text figure 2 is given a camera lucida drawing illustrating various of these structures.

The sclerotia formed so abundantly on malt agar by strain E–33 are almost wholly lacking in strain L–34. They are formed in the former after 48–72 hours at 25° C., and after proportionate intervals throughout the temperature growth range of the organism. The sclerotia are extremely variable in size, ranging from 56 μ–203 $\mu \times 56$ μ–142.24 μ. They are formed from a single hypha or

TABLE 2

THE SIZE [4] IN MICRONS OF CONIDIA OF STRAINS E–33 AND L–34 OCCURRING ON MALT AGAR AND *Portulaca oleracea*

Spore classes	Medium	Strain E–33		Strain L–34	
		Range	Average	Range	Average
2-celled	Malt agar	None		15.30–22.95 × 6.38– 7.65	19.19 × 7.27
	P. oleracea	None		15.30–20.40 × 7.65	18.17 × 7.65
3-celled	Malt agar	20.32–40.64 × 10.16	30.78 × 10.16	20.40–38.25 × 7.65–10.20	27.92 × 8.05
	P. oleracea	25.50–38.25 × 7.80–10.20	31.65 × 8.90	22.95–28.05 × 6.63–10.20	24.34 × 10.04
4-celled	Malt agar	33.02–48.26 × 10.16	38.94 × 10.16	28.05–35.70 × 8.92–10.20	31.88 × 9.56
	P. oleracea	40.80–51.00 × 7.80–12.75	44.88 × 11.02	28.05–38.25 × 10.20–12.75	34.68 × 10.81
5-celled	*P. oleracea*	48.45–56.10 × 10.20–12.75	52.37 × 12.05	None	
6-celled	*P. oleracea*	50.55–56.10 × 10.20–12.75	54.82 × 12.75	None	

[4] The values are for 40 randomly selected spores in each spore class.

from a number of anastomosing hyphae, are irregular in shape, the surface black, the interior hyaline to dark gray. Each is composed of an aggregate of more or less symmetrical cells having

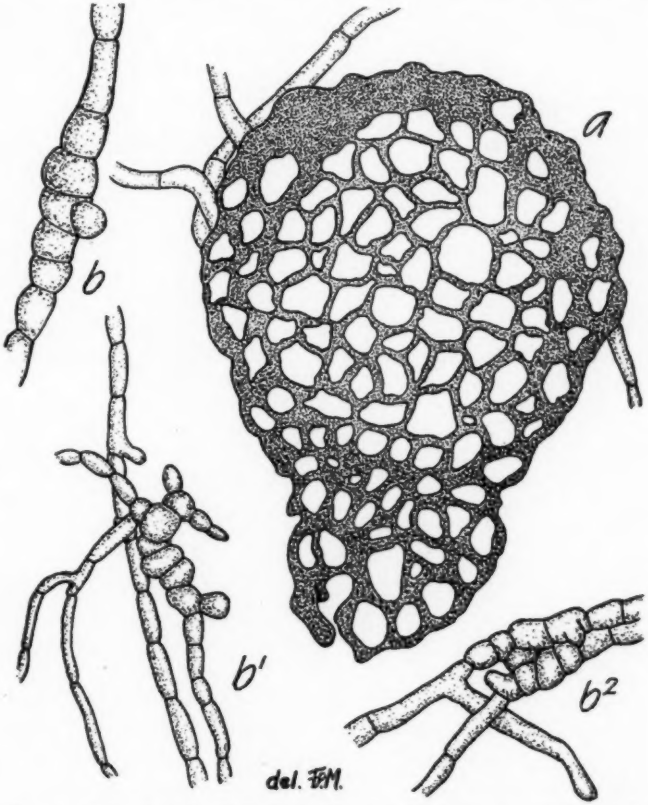


FIG. 3. Sclerotia of *Dichotomophthora Portulacae: a,* cross section through a mature sclerotium, × 500; *b,* various early stages in the formation of sclerotia, which may arise from a single hypha or by the fusion of several hyphae, × 500.

heavy walls. They germinate by germ tubes only. In text figure 3 is shown a cross-section of a sclerotium obtained with the freezing microtome, and a few early stages in the formation of sclerotia. All drawings were made using the camera lucida.

Dichotomophthora Portulacae gen. & sp. nov.

In the absence of a perfect stage, the characters detailed above place the organism in the division Macronemeae of the Phragmosporae, but its morphology does not agree with any described genus, nor does it approach any described genus closely enough to cause confusion. A new genus is therefore established, the formal description following:

Habitat: Biogenous on *Portulaca* species
Type locality: Hawaiian Islands
Description:

Mycelium copious, at first aseptate and hyaline, soon becoming septate, olivaceous to dark brown, anastomosing freely. Pycnidia and stromata absent. Conidiophores brown, regularly dicotomously to subdicotomously branched, successively elongating, 75–280 μ long $\times$ 5.0 μ in diameter. Terminal cells of the branches 4–8 lobed bearing a single conidium terminally upon each lobe. Conidia smooth, exogenous, one- to 6-celled, brown, ovoid to elongate-ovoid, rarely curved, 15–56 μ long $\times$ 6–13 μ wide. Sclerotia abundant, minute, black without, colorless to gray within, irregular in shape, 56–205 μ long by 56–145 μ wide.

Desiccated type-material has been deposited with the following universities: California, Chicago, Wisconsin, Cornell, and Harvard. It has also been sent to the Connecticut Agricultural Experiment Station, The New York Botanical Garden, United States Department of Agriculture, and the Imperial Institute of Mycology at Kew.

Cultures of the genus have been deposited at the Centralbureau für Schimmelpilzculturen, Baarn, Holland, and the McCormick Memorial Institute, Chicago.

NOTES AND BRIEF ARTICLES

MYCOLOGIA ENDOWMENT FUND

The Managing-Editor is pleased to announce the receipt of another gift which will be added to the Mycologia endowment fund held by The New York Botanical Garden. This gift was accompanied by the following note:

"I have just had some money come in, and it gives me *great pleasure* to make a (July 1) gift to Mycologia.

This is prompted in the main because of your superb contribution to the success of this magazine—and to be expended as your judgment directs."

This is the second gift of one thousand dollars made by the same person, the name of the donor being withheld by request.

It is the hope of the Managing-Editor that the interest on this endowment fund, when it has reached sufficient proportions, may be used to defray the cost of special features of the journal which cannot be carried on the regular income. The Managing-Editor takes this occasion to thank the anonymous donor for this gift.

I have a student making a taxonomic and morphologic study of the Patellariaceae. At the present time her work is greatly hampered by a lack of fresh material. We would greatly appreciate the coöperation of the members of the Mycological Society of America in sending to us any specimens of this family they may find. Mrs. Moldenke will also be glad to identify and annotate herbarium material. Specimens should be sent to me at The New York Botanical Garden.—Fred J. Seaver.

A New Monograph of Cortinarius

Professor Jacob E. Lange has recently published a monograph in English of the Danish Cortinarii in which he keys and describes 120 species being about twenty more than half the total number of North American species described in the N. A. Flora. He accepts the six Friesian subgenera and keys them separately dividing each subgenus into two subsections. The quality of such keys is always proved by their use; however, a careful reader of these six examples will probably admit the author's claim that they are so clear and plain that a "novice may be able to follow their lead without getting side-tracked on the road." The following gen-

eralizations occur in the interesting five-page introduction: the Cortinarii always grow on the ground. None of them are really xylophilous or coprophilous; comparatively few grow in grasslands and some inhabit moss but the majority are to be found in woods preferably in deep beds of foliage or rotten needles.

The monograph is published by the Dansk Botanisk Arkiv, Copenhagen. Pr. 10 kr. The fifteen colored figures will maintain the author's high reputation for color-work in this field. —JOHN DEARNESS.

CHRONICA BOTANICA

There are nearly 4,000 Institutions of pure and applied botany. There are between 60,000 and 70,000 botanists. There are about 1,000 periodicals concerned with botany! How can you keep in touch with all this activity? How can you find out what other botanists are doing and what new work they are planning. CHRONICA BOTANICA will help you. Subscribe to it and help with the compilation of the next volume.

All directors of institutions and secretaries of societies will receive a copy of our questionnaire at the beginning of December of each year. Replies should reach the Editor-in-Chief, Dr. F. Verdoorn, Leiden, Holland, not later than January 30th, as it will generally be impossible to make use of information received after that date. Directors or Secretaries, who do not receive our preliminary circular, which will reach them annually before Oct. 15th, are kindly requested to acquaint us of the fact at their earliest convenience, which will enable us to include them in our mailing list, and will ensure their receiving a copy of the questionnaire in December.

Prospectus, sample pages and further information may be had from the EDITORIAL AND PUBLISHING OFFICE, P. O. Box 8, Leiden, Holland.